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THIS NUMBER IS ISSUED

IN HONOUR OF

PROFESSOR

ALF SJÖVALL S

60TH BIRTHDAY

APRIL 19TH 1965

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PROFESSOR ALF SJÖVALL

ON HIS SIXTIETH BIRTHDAY

April 19th, 1965



A modern University professor in any branch of clinical medicine must have basic training and qualifications in one or more of the theoretical branches of medicine if he is to be able to direct the research and supervise the scientific work of his department.

Alf Sjövall, the doyen among active professors in obstetrics

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Alf Sjövall, the doyen among active professors in obstetrics

and gynæcology in Sweden extremely well fills these requirements It was not until after 2 years training in histology and four years work as a pathologist in the department of clinical pathology and forensic medicine that *Alf Sjövall* started his training in surgery obstetrics and gynæcology

Sjövall has published about a hundred papers on pathology obstetrics and gynæcology and on gynæcological urology Following an excellent thesis on the structure of the cervical mucosa in different periods of a woman s life supplemented by experimental investigations on guinea pigs with special reference to the influence of ovarian hormones on the function of the cervical mucosa and thereby on the migration of spermatozoa Alf Sjövall was appointed assistant professor of pathological anatomy in 1939 By 1943 his scientific efforts and ability were considered to warrant an assistant professorship in obstetrics and gynæcology The following year he was appointed to the chair of obstetrics and gynæcology in Lund becoming the chairman of one of Sweden s largest and oldest departments of obstetrics and gynæcology which was about to undergo considerable expansion and modernization It is mainly Sjövall who must be credited with the success of this reconstruction and above all for the amount of space devoted to research laboratories.

As a lecturer and a clinical teacher Sjövall is highly esteemed. He has modernized teaching methods and devised new ways of enabling the students to take a more active part in clinical work

Alf Sjövall is an upright and fearless man who will firmly and skilfully defend any opinion which he thinks well founded and just. He is a perspicacious diagnostician and a quick and neat handed surgeon He is also a patient listener with a gift for gaining a clear insight into delicate psychological problems Sjövall still has an inquiring mind and a burning interest in the new diagnostic and therapeutic methods His critical attitude and *sound common sense however keep* him out of exaggeration and mistakes

Alf Sjövall s gift for organization has found wide scope For many years he was president of Medicinska föreningen in Lund secretary and later president of Lunds läkaresällskap vice-*president and later president* of Sydsvenska gynekologsällskapet

and for eight years he was secretary-general to Nordisk förening för obstetrik och gynekologi. The Congress of Obstetrics and Gynaecology of the Northern Countries was held in Lund 1964 under his excellent presidency As the Editor of Acta Obstetricia et Gynecologica Scandinavica since 1960 Alf Sjövall has not only sacrificed much of his time and performed his duties in an admirable way but also put the journal on a much sounder economic basis.

This tribute to Alf Sjövall in celebration of his 60th anniversary would not be complete without mentioning, that in the eyes of the Fenno-Scandinavian gynaecologists of his generation he is a very good colleague, whose advice one willingly accepts, and at whose table one always feels a very dear guest.

Axel Ingelman-Sundberg

Bergman, S. Lundgren, K. M. and Lundström, P. Acta obst. et gynec. scandinav. 44, 8, 1965.
From the Departments of Clinical Bacteriology (Sven Bergman M.D.) and Gynæcology and Obstetrics (Professor Per Lundström) Umeå University Sweden

HÆMOPHILUS VAGINALIS IN VAGINITIS

A Study of a Cocci-dominated Vaginitis

BY

S. BERGMAN, K.-M. LUNDGREN AND P. LUNDSTRÖM

About 40 per cent of 200 consecutive patients complaining of varying degrees of vaginal discharge who attended a private gynæcological outpatient department at Umeå hospital suffered from vaginitis. Trichomoniasis was the most common type of vaginitis the second commonest type, pregnant and postmenopausal women being disregarded, was characterized by large numbers of cocci in the vaginal secretion. Further about 20 per cent of the 200 patients were diagnosed as suffering from chronic *cervicitis. In about a further 20 per cent the cause of the discharge* was obscure but slight vaginitis and cocci in the vaginal secretion characterized half the cases. Finally in 20 per cent the outstanding feature was a marked cytolysis of the epithelial cells associated with large numbers of Lactobacilli and probably a high vaginal acidity (Lundström and Petersohn 1963).

While the other groups were well defined the group with vaginitis associated with cocci were not examined in detail in the paper just mentioned. In this paper we have studied vaginitis characterized by cocci predominating in the cytological picture.

Methods

The specimens were always taken from the lateral wall of the vagina about one centimetre distal from the fornix, the pH values being measured afterwards at the same place.

Smears of solid material were made on glass slides and stained with methylene blue and also by Gram's method.

Table I. Age Civil Status and Parity

			AGE IN YEARS				
			<30	30—40	40—50	>50	Total
Case material	Nulliparous	single		4			6
		married		8			8
	Parous	single					1
		married		3	0	3	26
		Total	2	26	0	3	4
Control series	Nulliparous						22
	Parous						

Smears for cytological examination were stained with Harris haematoxylin-Shorr stain. In the cytological smears the eosinophilic index was calculated on all the epithelial cells and the pyknotic index from the eosinophilic cells only

The vaginal acidity was measured by flat glass electrode especially designed for surface pH measurements (Beckman electrode type 39182) connected with Beckman pH-Meter model 76

Material obtained for culture was put into four tubes with placenta broth at pH 4.0, 5.0, 6 and 7.0 From each of these tubes inoculation was made on placenta blood agar and on McLeod plates and incubated aerobically and anaerobically

Additional cultures were made in placenta broth and also anaerobically using thioglycollate and Tarozzi fluid medium. For isolation of Lactobacillus Rogosa-substrate was used The substrates were examined after 24, 48 and 72 hours From the fluid media films were stained by Gram's method and subcultures were made

Lactobacillus fermentation reaction was assessed according to Rosebury

Colposcopy was carried out in all cases.

Case Material and Control Series

The case material was selected as stated above and 1 other patients were added, selected according to the same principles.

From the time of the first examination of the patients, the results of which decided if the patient should be included in the series or not, there elapsed period of up to 6 months, though rarely exceeding two months, until the beginning of the investigation Thirty-six patients in the series were examined twice and 5 patients only once. Only the results of these examinations ar accounted for For further information see also heading of Table III

A control series for comparison consisted of 22 young women. Subjectively

Bergman, S Lundgren K M. and Lundström, P Acta obst et gynec. scandinav 44, 8, 1965

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While the other groups were well defined the group with vaginitis associated with cocci were not examined in detail in the paper just mentioned In this paper we have studied vaginitis characterized by cocci predominating in the cytological picture.

Methods

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Table III. *The occurrence of Lactobacillus and Haemophilus Vaginalis in the vaginal secretions in the different pH-groups. I all but 5 cases (these patients were examined at one occasion only) the patients were examined twice with an interval of one—two months or less without treatment in the interim.*

Demonstration by one or more methods of	pH-GROUPS			
	4 ←→ (N = 8)	5 ←— 6 (N)	←→ 6 (N = 2)	TOTAL (N =)
Lactobacillus	8	5	1	34
Haemophilus vaginalis	7	19	12	38

In the pH-group 4.0—> 6 the pH-values at the two separate examinations showed greater variation than in the other groups. Thus in 9 cases out of 24 examinations, with pH-values < 5.0 chiefly Lactobacilli were found in all but one case. When the pH-values were > 5.0 Lactobacilli dominated the microscopic picture in only 4 cases.
N = number of patients

Bacteriology. The bacteriological findings in the case material are evident from Tables III and IV. W would like to comment on some items. Lactobacilli and bacteria which, according to the cultural and smear findings belonged *to the Haemophilus* group, were found in the vaginal secretion of almost every patient. These bacteria even if encapsulated (G a d e e and D u k e s, 955) were considered to be Haemophilus vaginalis. Lactobacilli dominated the flora when the pH was < 5.0, but with decreasing vaginal acidity the number of Lactobacilli rapidly decreased as judged from the smear (For further information see Table III)

In 38 patients Haemophilus vaginalis was found in the vaginal secretion, while in only 3 patients these bacteria could not be demonstrated. Haemophilus vaginalis were seldom seen in great numbers in the cytological or Gram stained smears. As judged by culture, however Haemophilus vaginalis was sometimes the only bacteriological finding in spite of the fact that the smear was dominated by Gram positive cocci or Lactobacilli

The demonstration of Lactobacilli was preferably made by smear. For the detection of Haemophilus aginalis Gram stained smears and cultures were superior to cytological smear. This was especially *evident in the pH* groups with an acidity higher than pH 5 (not tabulated here). The identification of Haemophilus vaginalis in cytological smears is somewhat difficult and the microscopic examination should be *performed* using phase contrast (Figs. 2) Haemophilus vaginalis was almost always seen in Gram stained smears in those cases where it had been found by culture. Sometimes, however when the culture was negative, the bacillus was found in the Gram stained smear

Table II. *Duration of Vaginal Discharge in the Case Material*

	N
Uncertain	3
one year	3
1–3 years	11
3 years	24

N = number of patients

only 4 of these persons had a moderate discharge. (Initially 24 women were examined, but two were excluded as they suffered from trichomoniasis.)

The age, civil status and parity appear in Table I. In the case material most patients were of childbearing age. Only 14 of 41 women were nulliparous and 7 women were single. As is seen in the table the corresponding parameters in the control series differ significantly from those of the case material.

According to Table II most affected patients had had vaginal discharge for many years. Only one of the patients stated that her sexual partner had symptoms of discharge.

Results

A. *The Case Material*

No patient had symptoms other than those due to vaginitis.

When analysing the case material statistically the cases were divided into groups according to vaginal pH (Table III) and the findings are reported in accordance with this division.

Symptoms. In 68 examinations the symptoms of discharge were marked in only 5 cases, the patient complained of a moderate itching or burning of the vulva in 8 cases. At the other 75 examinations the patients stated the symptoms to be slight. Proportionately, the more severe symptoms were found as often in the groups with a vaginal pH < 5.0 as in those with a pH > 5.0.

Colposcopy. The colposcopic picture was either ordinary, or showed small ectopic areas or residues of a transformation zone. A definite reddening of the vagina was observed at 18 examinations only and was relatively more often found in the groups with a pH < 5.0. The discharge was never abundant and, in general, was relatively sparse. It was not foul smelling and varied in character.

Cytology. In the cytological picture the epithelial cells showed no significant changes. Generally, the number of leucocytes was rather scanty, only in 10 per cent of the examinations was a great number of leucocytes found, histiocytes were seldom seen. There was a tendency towards an increased number of leucocytes at higher acidity levels.

Table V *The bacterial vaginal flora of the control series determined by culture in relation to the findings in the cytological and Gram stained slides.*

	CULTURAL FINDINGS						
	L	HV	Bact–	Str	E	Y	Neg F
Cytology							
L only or most L, = 9	8			1			
Most C (+ HV in one case) =							
Most HV e =							1
Gram staining							
L only or most L, = 9	8						
Most C, e =							
Most HV e =		2					2

In 3 cases staphylococcus albus only was found
In cases either Gram neg or pos cocci were also found.

Haemolytic enterococci = E
Staphylococcus aureus = Y
For other abbreviations see Table IV

bacilli and streptococci were met with in about 45 per cent. Streptococci predominated over Gram negative bacilli, but the difference is small, 18 against 3.

B *The Control Series*

Table V shows firstly that again cytological and Gram stained smears are very similar and secondly that the most common finding was Lactobacilli. The difficulty of demonstrating Lactobacilli by culture is also evident in the control series. Only twice was Haemophilus vaginalis found. The vaginal bacterial findings were less complex than in the affected cases and this is perhaps most evident in the group of nonpathological bacteria where staphylococcus albus was found almost exclusively

No Listeria were found in these series. Cultures for PPLO *were not made.*

Discussion

The outstanding feature of the cocci vaginitis in question is as follows Signs and symptoms are in most cases slight, the vaginal acidity varies but is often low While the bacterial flora is very variable two bacilli are found throughout *viz.* Lactobacillus and Haemophilus vaginalis.

Table IV *The bacterial vaginal flora of the affected patients determined by culture in relation to the findings in the cytological and Gram stained slides. The figures within brackets indicate that in addition to Hæmophilus vaginalis, streptococci and/or Gram negative bacilli are present*

	CULTURAL FINDINGS			
	Only HV or HV + Str or/+ Rod—	Str or/+ Rod—	L	Non P
Cytology				
Most L, e = 27	12 (2)	4	9	11
Most C (+ HV in 19 cases) e = 50	27 (12)	10	17	19
Gram staining				
Most L (+ HV in 18 cases) e = 24	9 (2)	4	8	12
Most C (+ HV in 17 cases) e = 29	15 (5)	5	14	17
Various findings, e = 13	6 (3)	4	2	7

Number of examinations = *e*
Lactobacillus = *L*
Hæmophilus vaginalis = *HV*
Cocci (almost always Gram positive according to the Gram stained slides) = C
Gram negative bacilli (Escherichia coli or Proteus etc.) = *Rod-*
Streptococci (hæmolytic, anærobic, α- γ) = *St*
Non pathogenic organisms, (Subtilis, Diphtheroids, Sarcina lutea, Candida, Neisseria, Staphylococcus albus) = *Non P*
No growth = *O*

Table IV is a summary of the bacteriological findings in the affected cases. It is evident that the bacterial findings in cytological and Gram stained smears are similar It is true that identification of the bacteria is more easily and precisely done by Gram stained than by cytological smear and further Hæmophilus vaginalis is often overlooked cytologically Taking into consideration, Gram stained and cytological smears can simultaneously for the sake of brevity be related to the cultural findings.

When Lactobacilli dominated the smear implying relatively high vaginal acidity Hæmophilus vaginalis was found by culture in nearly 50 per cent, while Gram negative bacilli and streptococci were found in about 25 per cent. In the 50 cases with cocci predominating in the smear Hæmophilus vaginalis was found by culture in about 55 per cent, while Gram negative

Besides Hemophilus vaginalis and Lactobacilli two important groups of bacteria are found. The one group consists of Gram negative bacilli such as Escherichia coli, Proteus etc. and the other of streptococci of different types. β-haemolytic streptococci however were never found. Whilst the finding of Haemophilus vaginalis seems independent of the acidity level Gram negative bacilli and streptococci are found twice as often at a low vaginal acidity as is the case at higher acidity levels.

From the point of view of a causal relationship to the vaginitis it is difficult to evaluate the findings of the Gram negative bacilli and the streptococcal flora. It is evident, however that this flora in our affected cases is of a low pathogenicity and further in the vagina these bacteria may be considered contaminants. This is in accordance with Lash s findings (1954) as on an average the frequency of Gram negative rod like bacilli and streptococci in normal vaginae is of the same magnitude as that in our case material.

Our suggestion that the Gram negative bacilli and the streptococci found play no important part in producing the symptoms described is supported by the following fact. In trichomoniasis the bacterial flora is of a mixed type and similar to that found in our affected cases (Kessel and Gafford, 1940 Hite *et al* 1947 Lash, 1954 Pearl *et al.* 1956 and Burch *et al.* 1958) When treating trichomoniasis with Metronidazole an imidazol derivative (Cosar and Jolou 1959) this flora vanishes and the Lactobacilli return and become dominant within a few days (Durel *et al* 1959 Lundström 1961) in spite of the fact that Metronidazole which has strong trichomonicidal properties, has no great degree of antibacterial activity

When detailing Results we pointed out that the affected cases and the control series differ in many respects. This is especially evident as far as sexual behaviour is concerned, with many parous women in the affected cases and probably more sexual contacts as well. The bacterial flora was less contaminated in the control series than in that of the affected cases but, significantly Haemophilus vaginalis was very seldom found in the control series (2/22) and almost always in the affected cases (38/41) We feel that these two series which differ in age sexual habits and

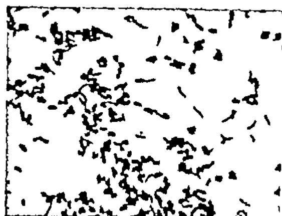


Fig 1 Gram stained vaginal smear × 1500. The thin rod-like encapsulated bacteria which are considered to be Hæmophilus vaginalis are seen in great numbers There are also a large number of cocci and a few Lactobacilli.

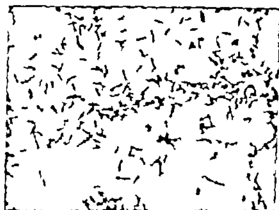


Fig. 2 Harris hæmatoxylin Shorr staining. × 1500. The picture corresponds well to that of the Gram stained slide.

The number of Lactobacilli is related to the pH When the pH is low Lactobacilli often occur in large numbers and vice versa Hæmophilus vaginalis however almost always occurs very sparsely Sometimes it may be found in large numbers but never in our case material forming clue cells as described by Gardner and Dukes (1955) Also for example like Heltai and Taleghany (1959) we never observed Hæmophilus vaginalis to be the only microorganism in the vaginal secretion

women being excluded—was characterized by large numbers of cocci in the vaginal secretion This coccal vaginitis group was examined bacteriologically and 11 other patients were added, selected according to the same principles. Thus our case material consists of 41 women.

While in most cases the vaginal acidity was decreased the other signs and symptoms were usually insignificant.

Bacteriologically the flora was of a mixed type but of a low pathogenicity As according to the literature the bacteriological findings in the normal" vagina are of the same kind as those in our series this mixed flora was considered to be a contaminant. Constantly in this coccal vaginitis series Lactobacilli were found as well as a bacterium which was considered to be Hæmophilus vaginalis.

The pathogenicity of Hæmophilus vaginalis is very difficult to evaluate but there are many facts and indirect evidence to suggest that Hæmophilus vaginalis is an important causal factor in vaginitis. It seems correct to state that in cases of vaginitis with a low pH in women of fertile age trichomoniasis being excluded a smear dominated by cocci strongly suggests the presence of Hæmophilus vaginalis.

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symptoms and signs obviously demonstrate that under quite different conditions Hæmophilus vaginalis occurs in the vagina with an entirely different frequency.

All these facts indicate that Hæmophilus vaginalis is an important causal factor in this type of vaginitis. But there is other indirect evidence too speaking in favour of this view. The one main cause of vaginitis the flagellate Trichomonas vaginalis, and Hæmophilus vaginalis have many epidemiological qualities in common. The same applies to the gonococcus as a cause for cervicitis. For instance all these organisms need very favourable condition as regards moisture and temperature.

There is therefore reason to state that our results seem to support the conclusions already drawn by Gardner and Dukes in 1955 as regards the pathogenicity of Hæmophilus vaginalis and the part these bacilli play as an important cause of vaginitis. We are proceeding with our investigation by way of therapeutic tests.

We would like to comment on some items concerning the diagnostic procedures. Culture of Lactobacilli and Hæmophilus vaginalis is no simple routine procedure. Typing of the Lactobacilli according to Tokazoe has given no uniform results. As regards Hæmophilus vaginalis it fulfils the criteria of the Hæmophilus group. In our investigation the microorganism is en-capsulated a fact which differs from the description by Gardner and Dukes. In a number of cultures no other bacteria than Hæmophilus vaginalis were found despite the fact that the smear was dominated by cocci. This may depend on the anærobic cultural conditions not being optimal.

Based upon our results in patients of fertile age and trichomoniasis being excluded it seems correct to state that in the case of a vaginitis with a low pH a smear dominated by cocci is strongly in favour of the presence of Hæmophilus vaginalis.

SUMMARY

About 40 per cent of 200 consecutive patients complaining of vaginal discharge suffered from vaginitis. The most common type was trichomoniasis, the other—pregnant and postmenopausal

women being excluded—was characterized by large numbers of cocci in the vaginal secretion. This coccal vaginitis group was examined bacteriologically and 11 other patients were added, selected according to the same principles. Thus, our case material consists of 41 women.

While in most cases the vaginal acidity was decreased the other signs and symptoms were usually insignificant.

Bacteriologically the flora was of a mixed type but of a low pathogenicity As, according to the literature the bacteriological findings in the normal vagina are of the same kind as those in our series this mixed flora was considered to be a contaminant. Constantly in this coccal vaginitis series Lactobacilli were found as well as a bacterium which was considered to be Hæmophilus vaginalis.

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AXIAL TORSION OF THE PREGNANT UTERUS

Report of a case

BY

EDV A. BJÖRKENHEIM

Axial torsion of the uterus is a very rare condition usually associated with pregnancy or intra-mural fibro-myomas (Glinski, 1910) Nordmeier (quoted by Hagblom, 1938) collected 45 cases of torsion of a pregnant uterus In 24 of them a large intra mural fibro-myoma was present. Twenty years later Nesbitt and Corner (1958) reported 107 cases of axial torsion of the pregnant uterus Single cases have also been reported, among others by Takatsky (1957) Ortiz (1957) Szlapak (1960) Resen Steenstrup (1960)

The rarity of axial torsion of the pregnant uterus prompted me to publish a case that came under my care

Case Report

The patient, a married nullipara aged 24 years was admitted to the Department of Gynaecology Deaconesses Hospital, in Helsingfors on the 17th June 1935, with severe pain in the lower abdomen.

Past history The patient had previously been treated in this hospital for right salpingitis. In April 1934, she was operated upon for sub-acute appendicitis. At the operation the body of the uterus was found to be regular in outline soft but not enlarged The adnexa were free from adhesions The menstrual periods had been regular but rather heavy and painful. The last menstrual period was on 14th April, 1935, and was scanty compared with her normal loss.

She had the habit of doing vigorous gymnastics every morning, and continued these exercises despite the fact that she had not menstruated for several months. She had been unduly tired and nauseated for some time prior

to her admission and during the preceding week she had had attacks of cramplike pain in the lower abdomen. There were no urinary tract symptoms or intestinal symptoms. She had been slightly pyrexial for two days. On the day of admission she awoke with severe pain in the abdomen and felt a large swelling above the symphysis pubis which she had not noticed previously

Findings on admission. The abdomen was distended, tense, and very tender A swelling in the lower part of the abdomen extended to a point about two fingers breadth below the umbilicus. There was no oedema.

The vagina was soft and congested. The portio was soft, of normal size and directed downward and backward. The body of the uterus was enlarged, it could not be differentiated from the swelling to the right of the uterus. The size of the swelling was compatible with five months pregnancy The tumour could be pushed upward as far as the umbilicus. Foetal heart sounds were absent. The diagnosis of a three months pregnancy complicated by a rapidly growing uterine myoma or torsion of an ovarian tumour was made. On account of the severe pain and meteorism laparotomy was performed on 8th June

Neither blood nor fluid was present in the abdominal cavity The swelling in the lower part of the abdomen was found to be large myomatous uterus twisted 90 to the left so that the right adnexa lay in the mid-line and in front of the tumour The latter was the size of newborn child head, was movable and was easily rotated back The adnexae were normal. The pain disappeared following the operation and did not recur The post-operative course was uneventful and the patient was discharged home on the eighth post operative day

The pregnancy proceeded normally foetal movements being felt for the first time towards the end of August On 20th January 936, she gave birth to a healthy female infant, weighing 2900 g. Delivery was uncomplicated. The placenta was expelled spontaneously

There was no noteworthy post-partum haemorrhage. The uterus did not decrease in size following the expulsion of the placenta and still extended to point about three fingers breadth below the umbilicus On 6th February mild haemorrhage occurred and this was associated with severe abdominal pain and slight pyrexia The patient was re-admitted to hospital She was anaemic The uterus together with the tumour which was hard and tender extended to point four-fingers breadth below the umbilicus. As the haemorrhage ceased she was discharged after two weeks in hospital. On 9th March she had another haemorrhage which was severe and associated with the passage of clots and severe pain The pulse was slightly irregular She complained of dizziness and undue fatigue. The sedimentation rate was 00 mm/ hour There was no pyrexia Episodes of haemorrhage recurred, the associated pain increased in severity and her general condition deteriorated. Removal of the tumour was therefore decided upon.

On 4th March, 936, the myoma was removed by subtotal hysterectomy The uterus was the size of newborn child' head.

Björkenheim Edv A. Acta obst. et gynec. scandinav 44, 18, 965.

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BY

EDV A. BJÖRKENHEIM

Axial torsion of the uterus is a very rare condition usually associated with pregnancy or intra mural fibro-myomas (Glinski 1910) Nordmeier (quoted by Hagblom 1938) collected 45 cases of torsion of a pregnant uterus In 24 of them a large intra mural fibro-myoma was present. Twenty years later Nesbitt and Corner (1958) reported 107 cases of axial torsion of the pregnant uterus Single cases have also been reported among others by Takatsky (1957) Ortiz (1957), Szlapak (1960) Resen Steenstrup (1960)

The rarity of axial torsion of the pregnant uterus prompted me to publish a case that came under my care.

Case Report

The patient, a married nullipara, aged 24 years was admitted to the Department of Gynæcology Deaconesses Hospital in Helsingfors on the 17th June 1935 with severe pain in the lower abdomen.

Past history The patient had previously been treated in this hospital for *right salpingitis. In April 1934, she was operated upon for sub-acute appendicitis* At the operation the body of the uterus was found to be regular in outline soft but not enlarged. The adnexa were free from adhesions. The menstrual periods had been regular but rather heavy and painful The last menstrual period was on 14th April 1935, and was scanty compared with her normal loss

She had the habit of doing vigorous gymnastics every morning, and continued *these exercises despite the fact that she* had not menstruated for several months. She had been unduly tired and nauseated for some time prior

recommend expectant treatment unless hæmorrhage or abortion occurs. In a few cases manual external correction of axial torsion of the uterus has been attempted but was successful in exceptional cases only (Löhlein 1910 Hitzanides, 1926 v Pall 1939) As in the case presented, Barzin (1959) and Resen Steenstrup (1960) corrected axial torsion of the uterus at laparotomy and allowed delivery to occur spontaneously

SUMMARY

A case of axial torsion of a myomatous uterus occurring in the third month of pregnancy is reported. The condition was not recognized prior to exploratory laparotomy The axial torsion was corrected and the pregnancy proceeded normally the patient being delivered at term of a healthy female infant. As the tumour increased in size and there were episodes of severe haemorrhage the uterus together with the tumour were removed by subtotal hysterectomy two months after delivery

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Histo-pathological report The tumour is a rhabdomyoma of the size o a newborn child's head and lies within the anterior wall of the uterus. Th uterine cavity is slightly distorted. The tumour is not encapsulated.

The post-operative course was uneventful and the patient was discharged home on 14th April.

Discussion

The clinical diagnosis of axial torsion of the pregnant uterus is difficult, and the condition is usually not recognized prior to laparotomy or post mortem examination Only some few cases of a correct pre-operative diagnosis have been reported (Löhlein 1910 Kiparsky 1924 Nürnberger 1930) Most often the torsion occurs near term or at delivery and only a few cases have been encountered in which torsion occurred as early as in the presented case that is between the second and fourth week of pregnancy (Küstner 1930 v Pall 1939 and others) Most authors agree that the uterus usually rotates from left to right, but a few cases have been reported (Kiparsky 1924 Hagblom, 1938) in which the rotation occurred from right to left as in the presented case According to Vogel (1940) however rotation to the right and left is equally frequent.

The discussion of the ætiological factors in axial torsion of the uterus is beyond the scope of this paper but I would like to mention those which Sellheim (1922 1930) considered to be of importance because they may be the most plausible namely a sudden twisting of the body when lying on the back, doing hard manual work, vigorous gymnastics or when dancing.

In the case presented here the patient did vigorous gymnastics every morning despite the fact that she was pregnant, a time at which the ligaments and the pelvic tissues are relaxed An additional ætiological factor was the intra mural myoma which was not evident at the laparotomy carried out a year previously and which had since grown enormously

At the laparatomy it was impossible to remove the tumour without interfering with the pregnancy hence the abdomen was closed hoping that the pregnancy would proceed normally The treatment of axial torsion of the pregnant uterus has been lively discussed. Hitzanides (1926) Hofmeier (1928) and others

Case Material and Methods

The total case material comprised 22 women in the 17th–20th weeks of pregnancy Two series of arteriograms were carried out, one during uterine contraction and the other during relaxation. The oxytocin group totalled 13 and the saline group 9 patients. Oxytocin was infused intravenously in doses ranging between 50 and 500 mU per minute and the arteriographic studies were carried out when regular uterine contractions had been obtained. In the saline group 150–250 ml amniotic fluid was withdrawn by abdominal puncture and approximately the same amount of 20 % sodium chloride injected. The arteriographic study was carried out 16–20 hours later when strong regular contractions were present.

The arteriographic technique has been described in detail earlier and involves percutaneous puncture of one of the femoral arteries, introduction of a polythene catheter into the aorta and injection of a contrast medium followed by a rapid sequence of roentgen exposures (Borell and Fernström, 1961 Fernström, 1955) A volume of 70 ml of contrast medium (60 % Urografin) was injected during a period of 4 seconds. The film frequency corresponded to one exposure per 1 5 second and there were 15 exposures in each series. The total dose of irradiation received by the gonads was calculated as approximately 1–3 r

Uterine motility was recorded by measuring the amniotic pressure (Caldeyro-Barcia and Alvarez, 1952) The contrast medium was injected at the middle of the ascending phase of the contraction wave because the circulatory retardation was found in the earlier study to be most marked at this moment.

Results

The maximum amniotic pressure during contraction was on an average 50 mm Hg in the oxytocin series and 57 mm Hg in the saline series. Uterine tone was normal, *i.e.* 12 mm Hg, except in three cases in the former and three cases in the latter group where the tonus of the uterus was moderately elevated.

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AN ARTERIOGRAPHIC STUDY OF THE BLOOD FLOW THROUGH THE UTERUS AND THE PLACENTA AT MIDPREGNANCY

BY

ULF BORELL, INGMAR FERNSTRÖM, LARS OHLSON AND NILS WIQVIST

An arteriographic study of the influence of uterine contractions on the blood circulation through the human uterus and the inter villous space has recently been published (Borell Fernström Ohlson and Wiqvist 1964) The study was carried out on women in midpregnancy admitted to the hospital for therapeutic abortion Uterine contractions were induced by intravenous infusion of oxytocin In order to obtain strong uterine contractions the oxytocin doses had to be kept at very high levels Such large oxytocin doses may influence the myometrium as well as the muscle fibres of the utero-placental vessels unphysiologically and hence give an incorrect picture of the circulatory events

The present investigation of the utero-placental blood circulation was carried out by the same technique as that used in our previous study the difference being that uterine contractions were induced by intraamniotic injection of hypertonic saline

On comparing the data obtained from the oxytocin induced cases with those of the saline-induced there turned out to be no significant differences between the groups This fact permitted pooling the data for analysis Thus the combined series of 22 patients offered the opportunity to give a more complete and quantitative description of the circulatory events associated with uterine contractions at midpregnancy

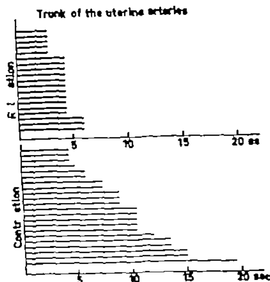


Fig. Midpregnant women admitted for legal abortion. Graphic illustration of the period of time during which the trunk of the uterine arteries was visible after intra-aortic injection of the contrast medium. Abscissa time in seconds. Injection of the contrast medium started one second before zero time. The horizontal bars represent individual cases and have been arranged in accordance with the appearance and disappearance of the dye. Some of the patients were studied only during uterine contraction.

when comparing the corresponding values for the intramural arteries. During relaxation the contrast medium appeared in the intervillous space at approximately the same moment in both groups. During contraction the intervillous space was only partially and faintly filled with dye in both groups. Dye appeared in the space in only 4 of 13 oxytocin cases and 6 of 9 saline cases. The mean intervals between injection of dye and appearance in the intervillous space were 10.9 and 6.3 seconds respectively. This difference might indicate a more marked retardation of the dye in the oxytocin group. The uterine veins filled with dye in 3 of 13 oxytocin cases and 2 of 9 saline cases. The intervals between injection of dye and appearance in the veins was 16 and 12 seconds respectively. Thus the difference in the degree of

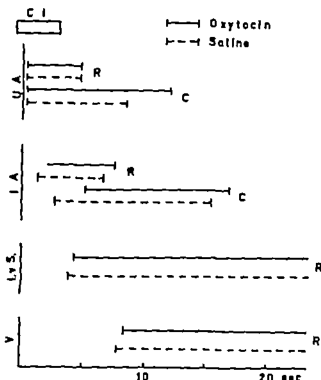


Fig. 1 Utero-placental blood circulation as studied by arteriographic technique *in therapeutic abortions. The figure illustrates a comparison between cases* with oxytocin induced uterine contractions and contractions induced by intra-amniotic injection of hypertonic saline. Oxytocin series mean values from 13 cases. Saline series mean values from 9 cases. The solid and dotted lines indicate the time during which contrast medium remained in the various vascular areas after injection of the dye C I, contrast injection U A, main branch of the uterine artery I A intramural arteries Iv S intervillous space, V uterine veins R, uterine relaxation C, uterine contraction. During contraction the intervillous space and the uterine veins were only partially visualized and therefore the time during which they were filled is not noted in the figure.

Fig 1 shows the mean values for the moment of appearance and duration of dye in the trunk of the uterine arteries the intramural branches the intervillous space and uterine veins. The time interval during which the trunk of the uterine artery could be seen was almost the same in both series although the dye stayed a little longer during uterine contraction in the oxytocin cases There was no definite difference between the two series

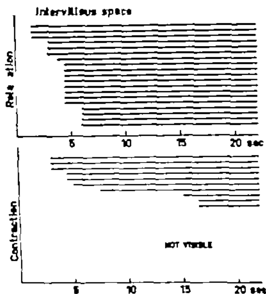


Fig 4 Same as Fig but for intervillous space.

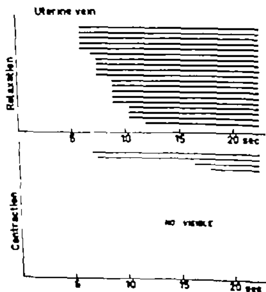


Fig 5 Same as Fig but for uterine veins.

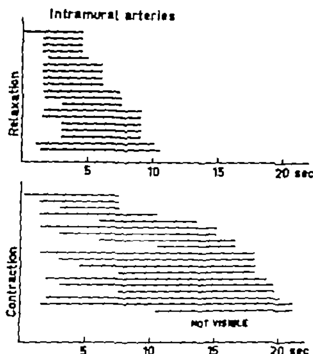


Fig. 3. Same as Fig 2 but for intramural arteries.

retardation between the oxytocin and saline series was very small and insignificant.

The similarity of the results obtained from the two experimental series justifies combined analysis of the data

The progression of the contrast medium within the various vascular areas of the uterus is illustrated in Figs 2–5. The average values obtained were as follows During relaxation as well as contraction dye appeared in the trunk of the uterine arteries almost instantaneously after injection of the dye and stayed there for 4 3 and 9 7 seconds respectively (Fig 2) The intramural arteries became filled with dye after 1 7 seconds (relaxation) and 3 9 seconds (contraction) and the corresponding duration of the dye within these arteries was 5 4 and 11 9 seconds (Fig 3) During uterine relaxation the contrast medium reached the intervillous space after 4 1 seconds and remained there for the rest of the observation time (20 seconds) During contraction dye could be found in the space in only 45 per cent of the patients and in these

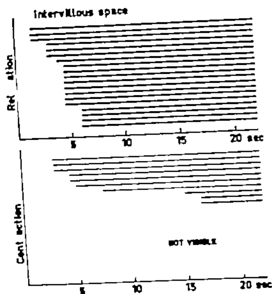


Fig 4 Same as Fig. but for intervillous space.

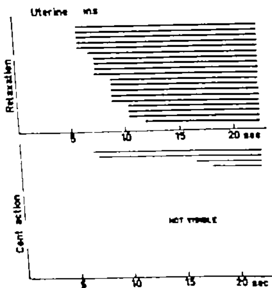


Fig. 5 Same as Fig. but for uterine veins.

Table 1 *Influence of the Intensity of Uterine Contractions on the Contrast filling of Various Vascular Areas*

Contractions	No. of Cases			
	Total	Intramural Arteries Visible	Intervillous Space Visible	Uterine Veins Visible
below 50 mm Hg	13	12	7	5
above 50 mm Hg	9	7	3	0

cases only a limited number of intervillous space entries could be seen The amount of dye within the individual entries was also markedly reduced as compared with the corresponding entries during relaxation (Fig 4) The uterine veins filled with dye 8.2 seconds after the injection during relaxation and the contrast medium stayed there for the rest of the period observed. During contraction small amounts of dye could be seen within the veins in only 23 per cent of the cases (Fig 5)

The relation between the intensity of the contractions and the degree of circulatory retardation is illustrated in Table I It appears that no veins became filled if the amniotic pressure exceeded 50 mm Hg whereas veins could be seen in 5 out of 13 cases if the pressure was less than 50 mm Hg There was a similar tendency with regard to the intervillous space although less evident. These results indicate that the circulatory retardation was more marked during strong than during weak contractions as would be expected. However the difference was comparatively small Some cases with low amniotic pressures showed marked inhibition whereas others with high pressures exhibited less retardation of the blood flow

Discussion

According to Csapo the myometrium overlying the placenta should be more or less completely blocked against propagated contractions whereas the extraplacental segments of the uterine wall should be only partially blocked (Csapo 1961) Such a functional asymmetry of the myometrium might also influence the blood flow through the uterus. Intra-amniotic injection of hypertonic sodium chloride at midpregnancy results in strong

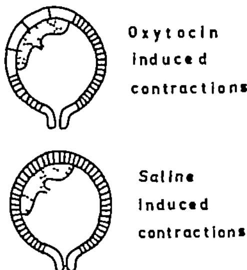


Fig. 6. Schematic drawing illustrating the hypothetical background of the experiment shown in Fig. Oxytocin induced contractions the striped extra-placental segment of the uterine wall indicates myometrium in active contraction, whereas the area over the placenta is considered as being relatively inactive due to the local placental block. Saline induced contractions: the entire uterus in active contraction due to suppression of the local placental block by intra-amniotic injection of hypertonic saline.

uterine contractions after approximately 16–20 hours and expulsion of the foetus 34 36 hours after the injection (Bengtsson and Csapo 1962 Wiqvist and Eriksson, 1964) The activation of the uterus should be due to a suppression of the placental function and thereby elimination of the placental block.

Although many experimental results indicate the existence of a local placental block of the myometrium conclusive evidence, based on studies of the contractile behaviour of the human uterus is still lacking. The present study is primarily devoted to the utero-placental blood circulation. However the design of the experiments also offered the opportunity to find out whether or not a possible uterine asymmetry would be reflected in the circulatory events. The hypothetical background of this idea is illustrated in Fig. 6 Although the dye possibly was somewhat

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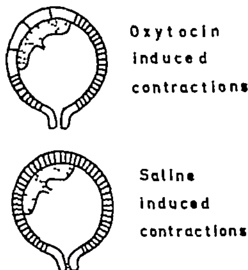


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less retarded within the intervillous space in the saline series the results obtained do not indicate any evident difference between the oxytocin series and the saline series. Even if it is assumed that the two model experiments represent asymmetric and symmetric uterine activity respectively it is still difficult to predict the influence of uterine contractions on the circulatory events. Thus, it is not known whether active contraction of a certain segment of the uterine wall impairs the blood flow more than does passive stretch

In both categories of patients uterine contractions were induced by unphysiological methods. The conditions are therefore not identical with those in normal labour at term. However the circulatory retardation associated with uterine contractions is of the same character as that observed by Ramsey *et al* (1960 1962 1963 a and 1963 b) in experiments with the rhesus monkey at various stages of pregnancy and labour We have also carried out a similar study in a limited number of pregnant women with malformed foetuses at term. The results in these cases were virtually identical with those in midpregnancy

An analysis of the material available does not elucidate the detailed mechanism or mechanisms responsible for the circulatory retardation *i e* whether or not the main hindrance is localized to the intramural arteries the spiral arteries or the veins. However in accordance with Ramsey *et al* our data show that some arterial branches and some parts of the intervillous space become filled with dye during contraction whereas others do not. This observation indicates the existence of local factors. A local retardation of the blood flow may be due to external compression of the arteries or veins by a sphincter like action of the twisted myometrial bundles The existence of such a mechanism presupposes that the arteries are influenced more by active contraction of the myometrium than by passive stretch Some authors maintain that the intramyometrial pressure during uterine contraction is higher than the amniotic pressure whereas others have found virtually identical pressures. The experiments of Ramsey *et al.* indicate that the muscle fibres within the arterial walls may constrict the vessels since segmental constriction may be seen as well during uterine relaxation

The studies on pregnant patients at term showed some intra mural arteries with one-sided impressions indicating external compression probably caused by some spiral or sling-like arrangement of the myometrial bundles. There was an approximate although not absolute, correlation between the intensity of the contractions and the degree of circulatory retardation These observations, as well as the fact that the retardation of the blood flow occurs synchronously with each contraction, seem to be most compatible with the view that the muscular events within the myometrium play an important rôle in retarding the blood flow during uterine contractions.

SUMMARY

Uterine contractions at midpregnancy induced by oxytocin or hypertonic saline caused a marked retardation of the utero-placental blood flow This retardation was well demonstrated in the intramural arteries as well as in the intervillous space and the veins.

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HUMAN CHORIONIC GONADOTROPIN IN ABNORMAL PREGNANCY SERUM AND URINARY FINDINGS USING VARIOUS IMMUNOASSAY TECHNIQUES

BY

SAM BRODY AND GUN CARLSTRÖM

In the individual patient the severity of certain complications during pregnancy is difficult to appraise solely by clinical examination Signs and symptoms may be misleading, the clinical picture equivocal, and correct treatment accordingly uncertain. Thus during the first few months of pregnancy it is sometimes impossible to differentiate threatened from inevitable or incomplete abortion, and the diagnosis of missed abortion or of ectopic pregnancy may for a considerable period of time only be tentative In the later stages of gestation a *different group of complications* may make the choice of therapy difficult. Toxaemia of pregnancy diabetes and Rh-immunization are cases in point.

Most of these *conditions if serious* are associated with disturbances of placental function The endocrinological activity of the placenta is one of its functions which has attracted particular attention In general the physiological significance of this activity is still poorly understood, yet its diagnostic value has become increasingly apparent.

The endocrinological activity of the placenta exhibits two rather distinct phases During the first the production of HCG a protein hormone dominates the picture Considerable amounts of this

Abbreviations HCG human chorionic gonadotropin CF complement fixation PAI, passive agglutination-inhibition.

substance are then found in serum as well as in the urine During the second phase the steroidogenic activity of the foeto-placental unit attains impressive levels Particularly the urine is then flooded with steroids of placental origin and their metabolites.

The elaboration of rapid and accurate chemical procedures for the determination of oestrogens and pregnanediol has provided a means of assessing placental function. These indices however are most useful during the latter half of pregnancy During the early stages of gestation the impressive production of HCG has attracted more interest as a possible measure of placental function and efficiency

Lack of suitable methods rendered this approach difficult for a long time A number of bioassay methods that were relatively easy to perform yielded erroneous results due to lack of specificity On the other hand, specific and accurate procedures were too time-consuming and laborious to be of much value in clinical work.

In this situation it would seem that the introduction of immunoassay techniques for HCG might permit a more successful attack on these problems. The rapidity and precision of these procedures offer a solution to the methodological difficulties. Considerations however other than purely methodological ones, also contribute to the complexity of these matters, and questions relating to the value of urine and serum assays as an index of HCG production in the placenta become of prime importance.

In an earlier publication (Brody and Carlström, 1962) we presented some data on serum HCG levels in abnormal pregnancy during the first half of pregnancy This limit was set by the considerable scatter of HCG levels among individuals in the latter half of pregnancy with the frequent occurrence of very low normal levels. This paper is a continuation of these studies, the object being to analyse the value of HCG levels in serum as a measure of placental function and of the viability of the pregnancy In addition, simultaneous determinations of HCG in serum and urine from patients with pregnancy complications have been carried out by various serological techniques. Certain discrepancies observed prompted an analysis of the rate of disappearance of HCG from serum and urine after induced abortion.

Materials and Methods

Patients

The present investigation refers to patients suffering from various forms of disturbed pregnancy At the time of admission to the hospital all patients exhibited signs and symptoms of imminent abortion, and only those cases have been included in which at the time of the first examination and for subsequent periods of varying length the further course could not be predicted. In 23 cases the final diagnosis was threatened abortion and in all these patients pregnancy continued to term. In another 50 subjects the final diagnosis was inevitable or incomplete abortion, necessitating surgical intervention in the majority of cases. The clinical diagnosis was in all these instances corroborated by pathological analysis of material obtained at the instrumental exploration of the uterine cavity or in a small number of cases treated conservatively by clinical follow up Missed abortion occurred in three patients The clinical criteria adopted to establish this diagnosis implied proof that the abortus had been dead for at least two months. This was indicated by cessation of uterine growth or by actual diminution in size Ectopic pregnancy was diagnosed in 17 patients.

The rate of disappearance of HCG from serum and urine was investigated in a group of patients where legal abortion was performed. In three patients abortion was induced by means of intra amniotic injection of hypertonic saline In one subject abdominal hysterotomy and sterilisation were performed.

Collection of Specimens

Blood samples were obtained by venipuncture and the serum was separated and inactivated at 56 C for 30 minutes. All samples were stored at −20 C Urine samples were collected without any preference for morning specimens

Antigen

Gonadex (Leo Hälsingborg Sweden) was used for the immu

nization of rabbits and for coating sheep erythrocytes. The inter national HCG standard preparation was employed as a reference

Antisera

Antisera to HCG were prepared as reported earlier (Brody and Carlström, 1961 1962) The effects of non-specific antibodies were eliminated in accordance with methods described in detail (Brody and Carlström, 1960 1961 1962) In the CF test 4 to 8 antiserum units were used (Brody and Carlström, 1961) This usually corresponds to a dilution of 1/32. The anti serum dilution employed in the PAI test prepared in the laboratory was usually 1/2000.

Serological Techniques

The CF procedure employed has been described in detail (Brody and Carlström, 1961 1962) This method was used exclusively for immunoassays on serum. For the determination of HCG in urine several PAI techniques were used. A haem agglutination-inhibition test was prepared in the laboratory Sheep erythrocytes formalinized and tanned as described by Weinbach (1958 1959) and modified by Wide (1962) and coated with HCG as reported by Wide (1962) were used for urinary estimations. In addition to this three commercial PAI tests were employed, namely Pregnosticon (Organon) Prepuerin (Burroughs Wellcome & Co.) and Gravindex (Ortho Pharmaceutical Cor poration) The sensitivity of the CF test was two IU of HCG per ml and that of the laboratory PAI test was in the same range The sensitivity of the commercial preparations was not tested.

Results

Quantitative Analysis of HCG in Serum

The patients are grouped according to the final diagnosis. On admission all subjects with intrauterine pregnancies were classed as having an imminent abortion, no differentiation between threatened and inevitable or incomplete abortion by means of clinical examination being possible in these cases. The amount

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Inevitable or Incomplete Abortion. In 15 patients of this category a negative reaction for HCG in serum was obtained. In another 35 cases HCG levels were found that fell below the lower limit of the 95 per cent confidence interval once or repeatedly. Some patients had HCG levels that were initially within the normal range but repeated analyses soon revealed a very rapid downward trend in the serum HCG concentration. Finally in a few patients, repeated examination showed normal HCG levels. Fig. 1 illustrates these findings.

Missed Abortion. In the period covered by this study three patients were diagnosed as having missed abortion. In one case a definitely subnormal level was found a few days prior to the spontaneous expulsion of the abortus. In the other two patients negative reactions for HCG in serum were observed three to four months before final evacuation of the uterine cavity.

Ectopic Pregnancy. In 8 patients negative reactions were found. In the other 9 cases the HCG levels were subnormal or showed a very rapid decline from normal levels. The findings in the latter group of patients are indicated in Fig. 1.

Simultaneous Analyses of HCG in Serum and Urine

In all the above patients where quantitative CF tests were performed on serum, qualitative assays were also carried out on HCG in the urine with a variety of PAI tests.

In 84 patients the results found in serum and urine were the same. In nine subjects, however, these results were conflicting. The clinical and laboratory data have been condensed in Table I. It will be seen that in these cases the reaction for HCG in serum became negative earlier than in urine.

Rate of Elimination of HCG from Serum and Urine after Induced Abortion

In four patients where legal abortion was performed reactions for HCG in serum and urine were carried out by the CF test and by the PAI test prepared in the laboratory, respectively. The results are summarized in Fig. 2. As may be seen, the reaction

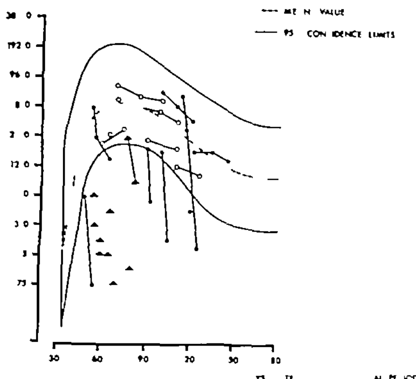


Fig 1 Immunoassays of HCG in serum by means of CF in patients with threatened abortion (open dots) inevitable or incomplete abortion (small black dots) and ectopic pregnancy (triangels) Interconnected points indicate serial assays on same patient. Mean value and 95 per cent confidence limits for normal HCG pattern indicated.

of bleeding varied as did also the intensity of the pain. The cervical canal and particularly its internal orifice were however invariably closed. The duration of symptoms before admission ranged from a few hours to several weeks

Threatened Abortion The results of the HCG analysis in this group of patients are shown in Fig. 1 and are represented by open dots. The figure also illustrates the mean value and the 95 per cent confidence limits of HCG levels in serum. This pattern and its statistical delineations are based on single-specimen anal yses from 268 women all of whom had a normal antepartum course with the delivery of a normal baby All patients with the final diagnosis of threatened abortion had serum HCG levels within the normal range.

		bleeding. January 9 curettage. Pathological diagnosis: chorionic and decidual tissue. Clinical diagnosis: incomplete abortion						
6	I	Last period September 25. November 2 slight bleeding, gradually increasing. Admitted November 24. Uterus slightly enlarged, cervical canal closed. November 28 severe bleeding, curettage. Pathological diagnosis: pregnancy decidua, no chorionic tissue, no trophoblast. Clinical diagnosis: incomplete abortion	November 25 November 28	− −	+	+ +	+ −	+ +
7	II	Last period November 5. January temperature 39° C. Admitted same day. Uterus corresponding to size of 7 to 8 weeks gestation, cervical canal closed. January heavy bleeding and spontaneous expulsion of foetal tissue. Curettage same day. Pathological diagnosis: placental tissue with inflammatory reaction. Clinical diagnosis: incomplete abortion	January	−	+	+	+	+
8	I	Last period August 31. November 11 slight bleeding, gradually increasing. Admitted November 26. Uterus slightly increased in size, cervix closed. Symptoms subsided. December 2 heavy bleeding, curettage same day. Clinical diagnosis: incomplete abortion	November 27 December	− −	+ −	+ −	+ +	+ +
9	II	Last period July 22. November 3 considerable bleeding. Admitted same day. Uterus enlarged to size of 8 to 9 week gestation, cervix open to the finger. Curettage same day. Pathological diagnosis: decidual tissue. November 1 uterus enlarged as before operation, temperature around 38° C. Ectopic pregnancy. Hydatidiform mole? Chorioncarcinoma? Incomplete curettage? November 15 laparoscopy: enlarged uterus, no ectopic pregnancy. Continued bleeding. November 27 angiography: intrauterine pregnancy. Increased bleeding. November 30 repeated curettage. Pathological diagnosis: chorionic tissue. Clinical diagnosis: incomplete abortion.	November November 4 November 9 November 27	+ + −	+ + + +	+ + + +	+ + + +	+ + + +

1 = PAI procedure prepared in the laboratory; 2 = Pregnosticon; 3 = Prepurin; 4 = Gravindex.

Table. 1 *Clinical and Laboratory Data from Nine Patients with Conflicting Results of Serological Analysis of HCG in Serum and Urine*

Case No.	Gravidity	Clinical Data	Laboratory Data					
			Date	Serum HCG (CF)	Urinary HCG			
							3	
1	III	Last period Januari 25. Slight bleeding May 15 and 21 May 24 uterus enlarged to size of 10 weeks gestation, cervical canal closed. Since July 11 daily bleeding. Admitted to the hospital October 3 October 5 curettage Pathological diagnosis chorionic and decidual tissue Clinical diagnosis missed abortion.	May 24	—	+	+	—	—
			October 4	—	—	—	—	—
2	III	Last period September 4. Since November 1 daily bleeding. Admitted November 11 Uterus enlarged to size of 7 weeks gestation. Bleeding subsided. February 17 spontaneous expulsion of small foetus. Clinical diagnosis missed abortion.	November 11	+	+	+	+	+
			November 27	—	+	+	+	—
			December 15	—	—	—	—	—
3	I	Last period October 2 November 17 pain and bleeding. Admitted November 19. Uterus enlarged to size of 6 to 7 weeks gestation Cervical canal closed. Laparoscopy same day uterus somewhat enlarged and hyperemic, no ectopic pregnancy Continued bleeding. November 28 curettage Pathological diagnosis chorionic and decidual tissue Clinical diagnosis incomplet abortion.	November 19	—	+	+	+	—
			November 25	—	+	+	+	—
			November 28	—	—	—	—	—
4	V	Last period June 6 Bleeding August 30 September 19, 27 October 15 and 18 Uterus corresponded to size of 16 weeks gestation. Cervical canal closed. Symptoms subsided. Since October 25 bleeding and pain. Spontaneous expulsion of foetus November 2 Admitted to hospital same day curettage Clinical diagnosis incomplete abortion.	October 9	+	+	+	+	+
			October 25	—	+	+	+	+
			October 29	—	+	+	+	+
			November 2	—	—	—	—	—
5	II	Last period October 20. January 3 bleeding and pain. Admitted January 7 Uterus enlarged to size of 7 to 8 weeks gestation. Cervical canal closed. Increased	January 7	—	+	+	+	+
			January 9	—	+	+	+	—

In the present investigation a number of different immunoassay techniques were employed. The CF test was used exclusively for assays on serum. One reason for this is that in this test there is no need for extracting the HCG from the serum and therefore the analyses can be carried out directly on the sample. For the purpose of preparing specific antisera the saturation procedure was used as described earlier (Brody and Carlström, 1961 1962) The application of the CF test to analyses on urine necessitates absorption of most antisera (Brody and Carlström 1962) a procedure that causes some reduction of the specific antibody titre. For assays on urine the PAI tests seem suitable. These latter techniques on the other hand, are less well-adapted for the determination of HCG in serum, as the hormone has to be extracted from the serum before it becomes measurable.

In an earlier study we used the CF technique for the quantitative determination of HCG in serum in a number of patients with abnormal pregnancies (Brody and Carlström 1962) The results obtained in the present work confirm our earlier findings. The implications of these findings are that pathological pregnancies associated with subnormal HCG levels in serum have an unfavourable prognosis. On the other hand, patients exhibiting symptoms of an abnormal pregnancy but in whom the HCG levels fall within the normal range usually have a good chance of reaching term In a small number of such patients however spontaneous abortion occurred in spite of a normal level. These abortions as a rule took place rather late in pregnancy It is tentatively suggested that the aetiological and pathogenetic factors underlying these mostly late abortions associated with normal serum HCG levels are different from those usually prevailing during the first three or four months where subnormal HCG levels are found in the patients.

As may be seen in Fig. 1 some patients belonging to the group of inevitable or incomplete abortions initially had HCG levels within the normal range Repeated analyses within a short period of time revealed a rapid drop in the majority of cases. It is therefore suggested that such serial determinations be carried out in patients where the clinical picture remains obscure.

The conclusion to be drawn from these findings and our earlier

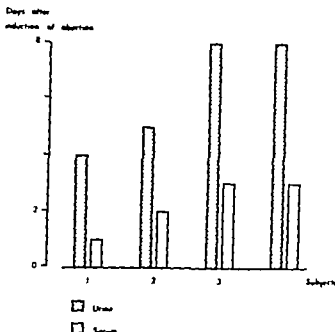


Fig. 2. Rate of disappearance of HCG from serum and urine after induced abortion. In case 1 the termination of pregnancy was performed by abdominal hysterotomy in the other cases by intra-amniotic injection of hypertonic saline

for HCG in serum became negative two to five days before a negative reaction in the urine was found

Discussion

It has long been known that a decrease in the HCG level in serum or urine to subnormal values indicates a disturbance in placental function and gives warning of an unfavourable outcome of the pregnancy Rakoff (1940) appears to have been the first to call attention to this relationship and the value of quantitative assays of HCG for the diagnosis of intra-uterine fœtal death during the first half of pregnancy has been repeatedly confirmed (Delfs and Jones 1948 Szejnberg and Rabau 1950 Rabau and Szejnberg 1955 Hon and Morris 1955 1956 Delfs 1957 Zondek and Goldberg 1957 Vermelin Ribon and Ribon 1957) Owing to the considerable technical difficulties involved in the employment of bioassay methods the clinical application of this index of placental efficiency has not become a routine procedure

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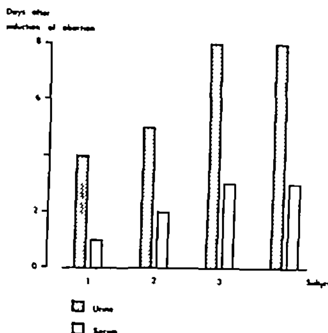


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present study. Rigid clinical criteria for the selection of the case material are necessary as the inclusion of clinically recognizable cases of inevitable or complete abortion will considerably distort the results. Only a simultaneous comparative study on serum and urine from the same patient after careful consideration of clinical data will yield reliable information.

SUMMARY

The value of immunoassay of human chorionic gonadotropin in abnormal pregnancies during the first half of pregnancy has been analyzed.

The quantitative determination of the hormone in serum by means of complement fixation yields information of considerable prognostic value. Subnormal HCG levels are associated with an unfavourable outcome of the pregnancy whereas HCG levels within the normal range usually imply a good prognosis. Such analyses permit a choice of conservative or active therapy at a considerably earlier period than would be possible on the basis of clinical examination.

Parallel immunoassays of HCG in serum and urine from patients with abnormal pregnancies by the complement fixation test and by various passive agglutination-inhibition procedures respectively gave in some cases discrepant results. Reactions for HCG in serum sometimes became negative before this occurred in the urine. In general the same trend was found in patients in whom abortion was induced.

The implications of these findings are discussed.

ACKNOWLEDGEMENTS

This investigation was supported in part by a grant from the Swedish Medical Research Council.

Our thanks are due to Burroughs Wellcome & Co. and to Ortho Pharmaceutical Corporation for generous supplies of Prepuerin and Gravindex, respectively.

results seems to be that quantitative immunoassay of HCG in serum during the first half of pregnancy is of considerable prognostic value in subjects where signs and symptoms of a disturbance of the pregnancy occur In patients where the pregnancy is not viable subnormal levels of HCG are found, irrespective of the localization of the ovum. In the light of such analyses treatment may be instituted from several days to several weeks earlier than can be done on the basis of clinical examination

As is apparent from the above results findings on serum and urine are not invariably in accord Thus in quite a few patients the reaction for HCG in serum became negative earlier than in the urine In this connection it should be emphasized that the sensitivity of the CF test was the same as that attained in the PAI test prepared in the laboratory The differing results cannot be ascribed to this factor

In principle the same result was encountered in a group of patients in whom abortion had been induced. The rate of disappearance of HCG from serum was considerably more rapid than that from urine

It is difficult to determine the reason for these divergent results. In this connection it should be mentioned that in general simultaneous bioassays of HCG in serum and urine yield the same results showing a considerably more rapid disappearance of HCG from serum than from urine (Behrman 1955) Whatever the explanation may be the practical implications of these findings are obvious The object is to find a parameter reflecting placental function in terms of its HCG production It would seem that the experimental data adduced favour the opinion that the serum HCG level is a more sensitive index of this activity than is the urinary HCG level

Comparative quantitative immunoassays of HCG in serum and urine were not carried out in this investigation No such analyses have yet been performed. The HCG level in urine from a number of patients with abnormal pregnancies was assessed by Wide (1962) and by Carlsson (1964) with a PAI procedure The results indicate a relatively good relationship between urinary HCG levels and the viability of the pregnancy It is difficult to compare the results of these two investigations with those of the

present study. Rigid clinical criteria for the selection of the case material are necessary as the inclusion of clinically recognizable cases of inevitable or complete abortion will considerably distort the results. Only a simultaneous comparative study on serum and urine from the same patient after careful consideration of clinical data will yield reliable information.

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LYMPHOGRAPHY IN CARCINOMA OF THE CERVIX

BY

M. EIKEN AND VALDEMAR MADSEN

The significance of lymphatic spread of cancer of the genital tract in relation to prognosis and therapy is well known. Until a few years ago radiologists had no means of establishing the exact extent of a carcinoma of the cervix but with the increasing recourse to surgery for this condition more exact information concerning the dissemination of such tumours is now often available. Even so the appearances at laparotomy may be misleading.

In our experience the extent of a carcinoma is commonly greater than is suggested by clinical examination whilst the reverse is rarely found. For this reason we now advise radiotherapy for all cases classified clinically as Stage II (II b) Prior to this we consider that too many patients with unrecognized advanced cancer were subjected to surgery We continue to operate on cases which clinically belong to Stage I or Stage I-II (II a)

The radiological visualization of the human lymphatic system has opened a new approach for improved classification of gynaecological cancers and for the recognition of recurrent disease. The first practical method of direct intralymphatic injection of water-soluble contrast media was developed by Kinmonth in 1954 The method was excellent for lymphatics but the nodes were only poorly visualized.

The introduction of an oily contrast medium, Lipiodol ultra fluid, syn. Ethiodol in U S A. (Wallace *et al.*, 1961 Sheehan *et al.* 1961 Rüttimann *et al.* 1961 Picard and Arvay 1961 and others) extended the usefulness of the method both by giving a more complete visualization of the lymph nodes especially of

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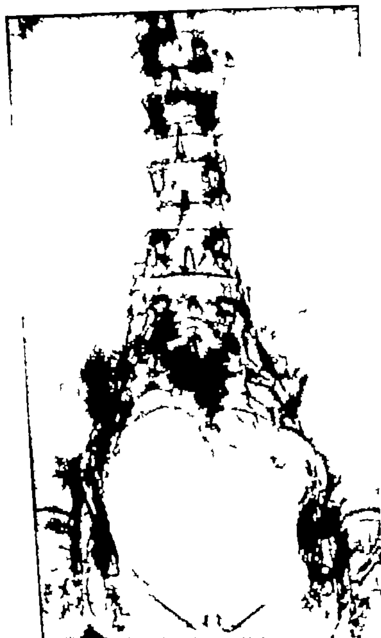


Fig Normal lymphangiogram immediately following injection.

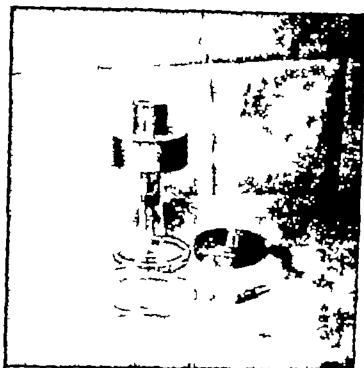


Fig 1 Injection stand with syringe connexion tube needle fitted to a thin nylon tube and three weights.

the retroperitoneal space and a more clear delineation of the structure of the individual nodes Since the contrast medium is retained in the nodes for 4 to 8 months a prolonged radiological study of the lymph node pattern is possible without further injection of contrast medium

A comprehensive review of the method and its diagnostic possibilities has been published by Rüttimann and del Buono (1964) while papers dealing especially with the value of lymphography in the field of gynæcology have been presented by Hahn *et al* (1963) Fuchs and Böök Hederström (1964) and Noriega L *et al* (1964) Furthermore the animal experiments by Tjernberg (1962) should be mentioned

Technique

The lymphographic method to outline the pelvic and retroperitoneal lymph nodes has been described frequently elsewhere



b



Figs. 3 a and b. Twenty four-hour left and right oblique films showing normal pelvic nodes (same patient as in Fig. 2)



b



a b

Figs 4 a and b Twenty-four-hour left and right oblique films showing normal para-aortic nodes (same patient as in Fig. 2)

It should be sufficient here to repeat briefly that the contrast medium is injected into an isolated lymph vessel on the dorsum of each foot. In our department the radioopaque medium was injected by a simple gravitational method, with a weight on the piston of the syringe. We did not find any advantages in the often used and more expensive electrically powered pumps. 8 to 10 cc of Lipiodol ultra fluid were injected on each side In most patients some of the contrast medium reaches the venous system by way of the thoracic duct. To reduce the risk of complications we

never exceed an injection speed of 1 cc per 10 minutes. Therefore the lymphographic examination usually takes 2 1/2 to 3 hours.

Roentgenograms are made at the completion of the injection to demonstrate the lymph vessels. 24 hours later, when the contrast medium is cleared from the lymphatics, additional roentgenograms give optimum visualization of the lymph node architecture.

When examining different lymphomas, it may be sufficient to take antero-posterior films of the pelvic and para-aortic nodes. However, to demonstrate small metastatic defects in the nodes, oblique films may be extremely valuable. In patients with carcinoma of the cervix, non-specific inflammatory changes in the pelvic nodes with slight enlargement and a reduced lymphatic flow are fairly common. In such cases, additional roentgenograms 48 to 72 hours after the injection may be necessary.

In this way the inguinal nodes, the external iliac group (frequently including the obturator node), the common iliac group and the para-aortic group can be visualized. A few nodes of the hypogastric group may also be demonstrated. The para-aortic group may be followed to the level of the 1st to 2nd lumbar vertebra. Thus the distribution of the radiopaque medium will on the whole correspond to the customary spread of gynaecological cancers.

Normal lymph nodes

The contrast medium is accumulated in the sinuses of the lymph nodes, giving each node a regular fine granulated and reticular pattern, well-defined from the surroundings. The number and size of the nodes may vary considerably, but although they may reach a size of 5 cm (Greening and Wallace, 1963) they rarely exceed 2–3 cm.

Some of the nodes show a small filling defect or indentation at the hilum. Larger radiotranslucencies may be caused by fatty infiltration or fibrous replacement. Finally, inadequate supply of contrast medium to the nodes may result in irregular opacification. Such non-specific findings may result in differential diagnostic difficulties (Fischer et al., 1962, Ditchek et al.,



Fig. 5. a) Twenty-four-hour preoperative lymphadenogram of patient with a Stage I b carcinoma of the cervix. The nodes are normal.



b) Postoperativ film with several nodes left behind despite an attempt t radical excision



Fig. 6. Roentgenogram of some of the nodes removed from the patient in Fig. 5. The architecture is normal.

1963). However, such filling defects occur mainly in the inguinal nodes, and even though they may be present in other groups of nodes it is felt that their presence does not seriously reduce the diagnostic value of the method.

Fig. 2 demonstrates the appearance of the normal lymphatics in a 40-year-old woman with a Stage I carcinoma of the cervix. The lymphatic channels can be seen to the level of the 1st lumbar vertebra, from which region it was possible to follow the passage through the thoracic duct. A slight degree of lymphatic obstruc-



Figs 7 and b Twenty-four-hour left and right oblique films of the para-aortic nodes, showing multiple lymphatic spread of carcinoma of the cervix. The filling defects are most numerous in the left latero-aortic group

tion in the pelvic region is not unusual in gynaecological patients and should not necessarily be deemed as abnormal.

In 24 hours the lymphatics have emptied almost completely (Fig 3) and the pelvic nodes belonging to the external and common iliac group are clearly outlined. Fig 4 shows the lymph nodes of the para-aortic group The nodes are all normal.

A radical operation has since been performed, and histological examination of the nodes confirmed the radiological diagnosis.

Lymphography performed prior to operation makes it possible to ascertain the effectiveness of a lymph node dissection. This is

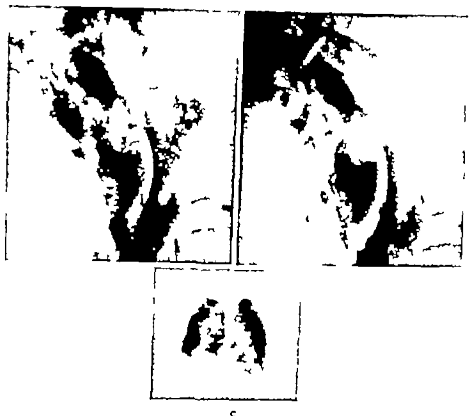


Fig 8. a) Lymph nodes of the left external iliac group before operation.
b) Same region postoperatively One node has been removed.
c) Roentgenogram of the removed node showing a large filling defect which represents a metastasis.

illustrated in Fig 5 To the left a 24 hours lymphadenogram of a 45 year-old woman with a Stage I b carcinoma of the cervix is shown The lymphographic pattern was negative and the diagnosis was confirmed histologically The accompanying roentgenogram was made after the operation and demonstrates that the node dissection was far from radical A roentgenogram obtained during the operation may certainly secure a more effective lymph node dissection Fig 6 shows a roentgenogram of some of the removed nodes from the above mentioned patient. The homogenous granulated and reticular pattern is clearly visible

Metastatic carcinoma

The nodal architecture following contrast opacification is partly characteristic, thus making it possible to differentiate between malignant lymphomas.

Metastatic carcinoma may in the early stage result in marginal filling defects, giving the nodes a moth-eaten appearance. There may be an increase in size but this is not always the case. When the node is partly replaced by tumour it may result in a sickle-shaped contrast shadow Fig 7 shows the nodes of the para-aortic group in a 44 year-old woman with a Stage III–IV invasive carcinoma of the cervix. Extensive lymphatic spread was demonstrated on the left side and surgical exploration confirmed the radiological diagnosis. Only a single node of the left external iliac group was removed for examination. Figs. 8 a and b show the region of the removed node before and after the operation and Fig. 8 c shows a roentgenogram of the node, which has been dissected for inspection. A large filling defect involving about one half of the node is clearly outlined, and represents a histologically confirmed metastatic carcinoma.

It should be stressed that defects in the nodes only reveal gross disease and especially in cases with solitary node irregularities great care must be exercised before interpreting the changes as specific A negative lymphographic finding does not exclude a microscopic spread of a carcinoma.

When there is complete replacement of the lymph node by tumour the node will no longer take up contrast medium. This is seen more often in cases of recurrent disease. In such cases, the node is usually enlarged and will often be outlined by displacement of the adjacent lymphatics and nodes.

In extensive metastatic involvement, complete obstruction of the normal lymphatic channels may be found, usually accompanied by filling of collateral lymph vessels. Figs. 9 and 10 show lymphograms at the completion of the injection and 24 hours later The patient was a 53-year-old woman treated 15 years previously by irradiation for a Stage I carcinoma of the cervix. During the following years she had been well until 4 months prior to the lymphographic examination when she complained of pain in the lower back and left leg. Intravenous urography showed

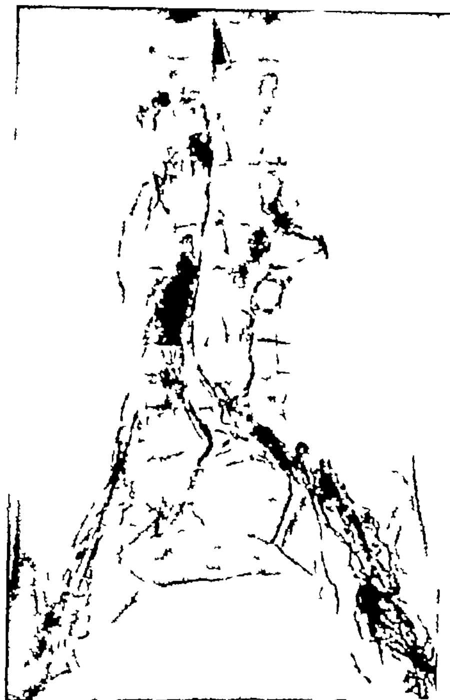


Fig. 9. Initial film of patient with recurrent disease. Note the marked *localized* displacement of the left latero-aortic lymphatics, thought to be due to an enlarged carcinomatous node.



Fig Twenty-four-hour film of same patient, showing the displaced normal nodes.

hydronephrosis Lymphography showed a marked displacement of the left latero-aortic lymphatics and nodes undoubtedly representing a grossly enlarged node completely replaced by tumour Such lymphographic results may be helpful in radiation therapy in order to achieve a more precise localization thereby allowing adjustment of treatment portals which will result in more effective and sparing irradiation

Chlorophyll-containing contrast medium

By incorporating chlorophyll in the contrast medium, the nodes which take up the medium will be coloured green thus facilitating their identification and removal during surgery Our experiments with this contrast medium are recent but seem promising

Complications

Lipiodol ultrafluid has already been used in so many examinations that it is commonly regarded as a safe contrast medium. Following lymphography it is usually possible to demonstrate a fine granular stippling throughout both lung fields representing a pulmonary oil embolism (Desprez Curely *et al* 1962 Bron *et al* 1963) However most patients have either mild symptoms or none at all The most common complication is a slight rise in temperature for 24 hours following the examination, occasionally accompanied by cough and pleuritic pain Severe reactions with hypotension transitory collapse hæmoptysis and cyanosis have only been reported by a few authors (Fuchs 1962 Bron *et al* 1963) Such reactions seem to predominate in debiliated patients possibly with impaired pulmonary function Too large an amount of contrast medium, and too fast an injection speed may contribute to the occurrence of untowarded reactions suggesting that an increased amount of contrast medium has bypassed the nodes and entered the thoracic duct and the venous system.

Conclusions

If a few simple precautions are kept in mind lymphographic examination may be a valuable help in the investigation and assessment of gynæcological cancers.

The following points should be emphasized

1. Lymphographic examination to detect metastatic carcinoma results in more exact assessment of the extent of cervical neoplasms.
2. Lymphography performed before during and after the operation may insure a more complete lymph node dissection.
3. The incorporation of chlorophyll into the contrast medium facilitates the recognition of the nodes during surgery
4. Negative lymphographic patterns do not exclude a lymphatic spread of tumour
5. On clinical suspicion of recurrent disease abnormal lymphography makes possible a more precise localization of the optimal portals of treatment.
6. As the contrast medium is retained in the nodes for a long time it is possible to evaluate the effectiveness of irradiation without further injection.
7. The examination should not be carried out on debilitated patients or on patients with acute or chronically impaired pulmonary function.

SUMMARY

Lymphography with an oily contrast medium was introduced only a few years ago but the method has proved to be a useful supplement in the investigation of a large number of pathological conditions, both of the lymphatics and of the nodes. The possibilities in the field of gynaecology especially in carcinoma of the cervix, are discussed. Although non-specific irregularities of the nodes may result in certain differential diagnostic difficulties it is felt that this method may contribute to the assessment of the extent of carcinoma of the cervix, and assist in the diagnosis of recurrences. During surgical treatment it permits control of the extent of lymph node dissection and in radiation therapy it facilitates a more exact localization of the treatment fields. Follow-up studies provide information as to the effect of irradiation The incorporation of chlorophyll in the contrast medium to facilitate the node dissection is mentioned.

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UTERINE MOTILITY AND CONCENTRATIONS OF PROGESTERONE IN UTERINE VENOUS BLOOD AFTER INTRA AMNIOTIC INJECTION OF HYPERTONIC SALINE

BY

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In 1962 Wagner Karker Fuchs and Bengtsson reported a series of 330 therapeutic abortions induced by intra amniotic injection of a hypertonic solution of sodium chloride. The authors found this method, which was first introduced by Aburel in 1934 very useful for interruption of pregnancy in the second trimester

When half or more of the amniotic fluid is removed and substituted by a 20 per cent saline solution abortion occurs after an average interval of 34 36 hours (Bengtsson and Csapo 1962 Wagner *et al.* 1962 Wiqvist and Eriksson, 1964) The frequency and intensity of uterine contractions increase appreciably after 6–10 hours as does the sensitivity of the uterus to exogenous oxytocin Strong uterine contractions can usually be recorded 12 18 hours after the injection of saline Often the

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Fig 1 Case No 20. Intra-amniotic pressure recordings. a. One hr after intra amniotic infection of hypertonic saline. Spontaneous activity and reactivity to 0.5 I. U of synthetic oxytocin (administered at arrow) b. Spontaneous contractions 19 hrs. after hypertonic saline. Progesterone concentration in uterine venous blood 18 μg/100 ml plasma. Time scale as in Fig 4.

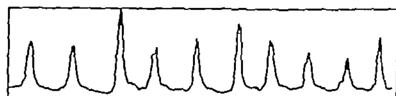


Fig 2. Case No 8 Intra-amniotic pressure recording. Spontaneous activity 13.5 hrs. after intra-amniotic injection of hypertonic saline. Progesterone *concentration in uterine venous blood 16 μg/100 ml plasma. Time scale* as in Fig. 4.

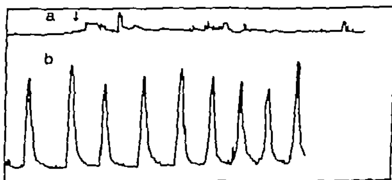


Fig. 3. Case No. 23. Intra-amniotic pressure recordings. a. Spontaneous activity and reactivity to 0.5 I. U synthetic oxytocin 20 min. after intra-amniotic injection of hypertonic saline. b. Spontaneous contractions 19.5 hrs. after hypertonic saline. Progesterone concentration in uterine venous blood 9 μg/100 ml plasma. Time scale as in Fig. 4.

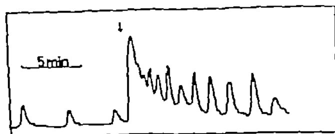


Fig. 4 Case No Intra-amniotic pressure recording. Spontaneous activity and reactivity to 0.5 I.U. synthetic oxytocin 5 hrs. after intra-amniotic injection of hypertonic saline. Progesterone concentration in uterine venous blood µg/ 00 ml plasma.

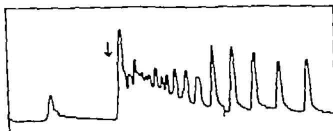


Fig. 5 Case No. 6 Intra-amniotic pressure recording. Spontaneous activity and reactivity to 5 I.U. synthetic xytocin 16.5 hrs. after hypertonic saline. Progesterone concentration in uterine venous blood 33 µg/100 ml plasma. Time scale as in Fig. 4

products of conception are expelled as a whole and the abortion is complete in more than half of the cases.

Intra-amniotic injection of hypertonic saline not only is an effective and comparatively safe method for induction of abortion t also provides an opportunity for studies which may shed some light on the mechanism of spontaneous abortion, and increase our knowledge of the factors responsible for the maintenance of pregnancy. Such studies were first carried out by de Watteville and his collaborators (Stamm and de Watteville 1954) and they have since been continued by several groups of investigators.

Bengtsson and Csapo (1962) maintain that the rapid increase of the uterine activity after intra-amniotic injection of

saline must be due to removal of the "progesterone block" of the myometrium as a result of placental damage. In the rabbit, the presence of a *progesterone block of the myometrium during pregnancy* has been demonstrated in extensive studies by Csapo and his collaborators and examination of the validity of this concept in human pregnancy would appear to be of great importance.

We have previously reported studies of the concentrations of progesterone in uterine venous blood in intact pregnancies (Fuchs *et al.* 1963) and after intra-amniotic injection of hypertonic saline (Short *et al.* 1965). The purpose of the present communication is to discuss the relation between the changes in uterine activity and reactivity and the concentrations of progesterone in uterine venous blood after saline injection.

Material and Methods

By means of intrauterine pressure recording the changes in uterine activity have been studied in a large number of cases. A detailed analysis of the records will be published elsewhere (Wagner, in preparation). Here we shall report the findings in 23 cases in which an abdominal hysterotomy was carried out at various periods of time after intra-amniotic injection of hypertonic saline and where uterine blood samples were collected for progesterone determination.

Withdrawal of amniotic fluid and injection of a 20 per cent solution of sodium chloride was carried out by the vaginal route with a needle inserted through the anterior uterine wall from the anterior fornix as previously described (Wagner *et al.* 1962). In 13 of the cases before withdrawal of the needle a polyethylene catheter was inserted and was left in place for intrauterine pressure recording.

The pressure recording was made with an Elema electromanometer and a Varian ink recorder. In some instances 0.5 I.U. of synthetic oxytocin (Syntocinon Sandoz) was given at intervals to observe the change in the reactivity to oxytocin. In intact pregnancies in the second trimester a dose of this magnitude provokes only a slight response, if any. The last recordings were usually made about 30 minutes before the laparotomy.

At laparotomy blood was withdrawn with heparinized syringes from the uterine wall at the placental side and from one of the uterine veins close to the uterus. Usually the placental site could be determined from the fact that the wall over the placenta bulges more than elsewhere. At the same time a sample of the peripheral venous blood was collected from a vein in the arm. The blood was centrifuged as soon as possible and the plasma was frozen and stored, and later shipped by air to Cambridge for progesterone determination, which was carried out as described by Short (1958) and Short *et al* (1965)

Results

The results are summarized in Table I With regard to the progesterone concentrations in the uterine venous blood, only the highest value in each case is recorded here regardless of whether the blood was obtained from the placental site or the uterine vein.

The progesterone concentrations in uterine venous blood of the saline treated women were lower than in the group of women with intact pregnancies studied previously (Fuchs *et al.* 1963) namely 24 1 vs 35.4 μg/100 ml plasma but the difference was only marginally significant ($0.01 < p < 0.05$)

An analysis of the progesterone concentrations in relation to the time interval between saline injection and blood collection (Short *et al* 1965) showed that the rate at which the progesterone level fell after saline administration was 0.71 ± 0.51 μg/100 ml plasma/hour but this regression was not significant.

It is evident from Table I that the spontaneous activity of the uterus and the reactivity to oxytocin was already increased 6–10 hours after saline when the progesterone concentrations were as high as about 60 μg/100 ml. Labour like activity was noted 13 hours after saline injection when the progesterone concentration was still 48 μg/100 ml (Case No. 7)

Figures 1 4 are examples of intrauterine pressure recordings They all show increased spontaneous activity or increased reactivity to oxytocin. This is in good agreement with results in the larger series of patients not subjected to laparotomy (Wiqvist

Table I. *The Uterine Activity and Uterine Sensitivity to Oxytocin in Relation to the Length of Interval after Intra-amniotic Injection of Hypertonic Saline and to the Progesterone Concentration in Uterine Venous Blood and Peripheral Blood.*

Case No.	Hours after saline	Gestation age weeks	Progesterone in uterine venous blood after saline (μg/100 ml plasma corr.)	Uterine activity increased	"labour"	Uterine sensitivity to oxytocin increased
1	2	15	20			
2	3	17	<5	+	o	
3	4	14	25			
4	6.3	22	59	+	o	+
5	10.5	19	61	+	o	+
6	12	15	18			
7	13	17	48		+	
8	14	13	16		+	
9	14.5	15	8	+	o	+
10	15	18	41			
11	15.7	19	21	+	(+)	+
12	15.8	16	32			
13	16	17	20		o	+
14	16.2	17	53			
15	17	14	37	?	o	
16	17.2	15	33	+	o	
17	18	17				
18	19.5	17	19		+	
19	19.5	22	1		+	
20	19.7	15	18	+	+	
21	20.5		30			
22	2	19	17		+	
23	21	24	9		+	

and Erikson 1964 Wagner in preparation) Usually there is a change in the uterine motility pattern as early as one hour after the saline injection The initial pattern is one of low amplitude and high frequency This gradually changes 4–10 hours after the saline administration into a pattern of high amplitude and lower frequency of contractions It is evident from the table that these rapid changes in the uterine motility are not coincidental with any appreciable changes in the progesterone levels in the blood coming from the placenta

Discussion

The mechanism of abortion after intra-amniotic injection of hypertonic saline is not completely known, but a number of factors are likely to play a role. The foetus dies very soon after the injection, probably due to dehydration The placenta is damaged (Bengtsson and Stormby 1963) which may influence its hormone production. The membranes are damaged and their permeability probably altered. The volume of the amniotic fluid increases on the average by 50 per cent during the first hours after injection, stretching the uterine muscle and thereby changing the mechanical properties of the myometrium. Sodium chloride diffuses out of the amniotic cavity and changes the ionic composition of the myometrium, which again may influence its mechanical properties The sodium and chloride levels of the plasma become elevated and cause a release of certain homeostatic factors which may include anti-diuretic hormone and oxytocin These and other possible factors have been or are subjects of investigation but even so their relative importance and their interaction will be difficult to evaluate Another important question is of course whether any of the above mentioned factors play a role in the mechanism of spontaneous abortion.

Intra-uterine pressure recording has proved to be a valuable tool in the study of uterine motility It is noteworthy that the motility pattern seen in the first hours after saline injection is different from that of spontaneous labour A period with high frequency and low amplitude is never observed in normal labour Gradually however a labour-like pattern develops. At the same time the response to a certain dose of oxytocin increases. This does not mean that only the sensitivity to oxytocin increases, it is more likely that the myometrium achieves an increased irritability in general.

The results of the present investigation indicate that the changes of the motility of the myometrium take place without any appreciable decrease in the progesterone output of the placenta. Whether there is a change of the progesterone level in the myometrium itself remains to be seen.

SUMMARY

In 23 patients in whom therapeutic abortion and sterilization was to be carried out abdominal hysterotomy and collection of samples of uterine venous blood was performed at various periods of time (2–22 hours) after intra-amniotic injection of 20 per cent sodium chloride The changes in the uterine motility were followed by intra uterine pressure recording The progesterone concentration was determined in the uterine venous blood and in samples of peripheral venous blood drawn at the time of operation and in some instances also before the administration of saline.

The results indicate that during the first 22 hours after injection of saline the motility of the uterus increases and changes into a labour like pattern These changes are not accompanied by any significant fall in the output of progesterone from the placenta

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CARCINOMA OF THE UTERINE CERVIX IN PREGNANCY

BY

GUNNAR GORTON

Malignant tumours in pregnant women have always created therapeutic problems. The most important question is Does pregnancy accelerate or retard the growth of the tumour? No unanimity has been achieved on this point. The situation is complicated by the fact that the mother as well as the child, and to a certain extent also the husband, must be considered. If the carcinoma is situated in the uterine cervix the gynaecologist may have to decide whether he should try to save the foetus despite the risks involved for the mother

According to the literature carcinoma of the cervix is detected in about one out of every 2000 to 10,000 pregnant women During the years 1947 to 1963 invasive carcinoma of the uterine cervix was diagnosed in my department in 11 women during pregnancy and in 9 during the puerperium. During the same period invasive carcinoma of the uterine cervix in various stages was diagnosed in more than 2000 non-pregnant women

In the diagnosis of carcinoma of the uterine cervix both clinical and historical data are important. Bleeding is the commonest symptom principally after coitus, and spontaneous bleeding without trauma Foul smelling vaginal discharge is usually a late symptom As to the clinical examination all women with vaginal bleeding during pregnancy should be examined gynaecologically The examination should include cytological examination vaginal inspection and palpation (which causes the patient practically no discomfort) It is now also the rule for all pregnant women

to be examined two or three times during pregnancy as well as about 6 weeks after parturition

Although treatment of pregnant women with carcinoma of the uterine cervix raises several therapeutic problems one thing is certain spontaneous delivery should never be allowed because of the serious complications it creates during parturition (hæmorrhage obstruction of descent of the child) and in the puerperium (hæmorrhage fever sepsis)

Carcinoma of the uterine cervix requires immediate treatment, which should be planned primarily to save the life of the mother The choice of treatment is dictated by the stage of pregnancy and the spread of the tumour In Lund during the first half of pregnancy we treat the patients in accordance with the following principles No attempt is made to save the fœtus. Radium treatment of the cervix and vagina is given and rœntgen radiation is used to kill the fœtus and thereby interrupt pregnancy Spontaneous abortion of a macerated fœtus usually occurs 3–4 weeks after treatment Removal of the regional lymph nodes is not considered until later

Treatment of carcinoma of the uterine cervix discovered during the latter half of pregnancy is different. If the carcinoma is in an early stage of growth we—unlike other authors—discuss the situation with the patient and her husband and give expectant treatment. The mother is first examined rœntgenologically for any malformations of the fœtus. She is then examined every third week and so far we have never observed any increase in growth of the tumour from one examination to another As soon as the fœtus is considered viable it is delivered by Porro Cæsarean section (classical Cæsarean section followed by high supravaginal amputation of the uterus with removal of the adnexa) Immediately after the operation the cervix and the vagina are treated with radium followed by conventional rœntgen therapy All the operations in our series were performed by Professor Alf Sjövall Department of Obstetrics and Gynæcology Lund.

In our opinion such patients should never receive radium treatment before delivery If the fœtal head presents the risk of injury to the head is considerable and may result in retardation of mental development or idiocy Radium treatment will produce

obstinate wounds on the buttocks of children presenting by the breech and may thereby retard the growth of the pelvic bones

We have applied these therapeutic principles in the treatment of 11 cases of carcinoma of the uterine cervix during pregnancy Six of the patients (all stage II) were in the first half of preg nancy The patients had symptoms for 2 weeks to 3 months before the tumour was discovered. One of these 6 women died after 3 years while 5 are still living (follow-up 4 years in one and more than 5 years in four) All the 5 women in whom the tumour was diagnosed in the latter half of pregnancy are still living (follow-up 1 2 5, 5 and 14 years after parturition) All of the children were normal (birthweights 2400–2900 g) In these cases expectant treatment was given for 1 to 4 months before delivery Caesarean section and irradiation were not at tended or followed by any complications. The regional lymph nodes were removed in all of the cases but in none was any evidence of metastases demonstrated.

During the 1947–1963 period 9 cases of carcinoma of the uterine cervix (Stages II or III) were discovered during the puer perium (in none had the growth been detected before delivery) The women had symptoms for 3 to 7 months before parturition. Three of the women died soon after commencement of treatment. The remaining 6 are still living (follow-up more than 5 years)

SUMMARY

At the Jubileumsklinik in Lund carcinoma of the uterine cervix discovered during pregnancy is treated according to the following principles. If the tumour is discovered during the first half of pregnancy the foetus is sacrificed to save the mother In cases diagnosed during the latter half of pregnancy the mother is given expectant treatment until the foetus is considered viable. The patient is then delivered by Porro Caesarean section and after wards treated by radiotherapy In our series treated in this way all the patients delivered by Caesarean section and their children survived.

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ACTIVE TREATMENT OF TOXÆMIA

BY

TEIT KÆRN

Toxæmia may be defined as a response of the body to pregnancy associated with endogenous and exogenous stress factors. In the extremely detailed, but still somewhat tangled mosaic of its pathogenesis the pituitary adrenal system is of particular interest. Graves and Agersborg phrased it in the following way Pregnancy *may* be regarded as a temporary forty week period of endogenous stress which is normally supported by increased activity of the pituitary adrenal axis It *may* then be supposed that in toxæmia this system somehow breaks down, but where this maladaptation starts and the why and wherefore of the hormonal changes so far remain beyond our perspective

From a pathophysical point of view the central feature of toxæmia is a universal arteriolar vasoconstriction resulting in changes in the blood flow especially in uterus placenta and kidneys

So much is certain the syndrome of toxæmia is seen in humans only and solely in connection with pregnancy later that the 24th week Furthermore it depends on functioning placental tissue Demographically we know that toxæmia occurs significantly more frequently in primigravidæ (three times the average frequency) in cases of twins and of polyhydramnios Furthermore about 25 per cent of women who have hypertension before pregnancy or at the beginning of pregnancy develop toxæmia

Clinical experience shows that there is a gradual transition from patients with a single abnormal sign such as œdema or hypertension through the mild to the severe cases of pre-eclampsia ending with eclampsia

It is an observed fact that more intensive antenatal care results in a favourable trend in the severity of cases. This is seen *inter alia* from the ratio between pre-eclampsia and eclampsia which in areas with poor antenatal care and a low social standard is about 15 1 while under average conditions it is about 30 1 and to-day in the best areas about 60 1

In principle treatment has not changed much during the last generation, although we have gained a better understanding of what we are doing and why Antenatal care results in earlier diagnosis and thereby the possibility of relieving stress, treating oedema by means of diuretics and increasing the uterine blood flow by bed rest. Anti-hypertensive drugs prove to be of no significant value in the treatment of pre-eclampsia but when administred as the "lytic cocktail" in eclampsia a considerably better prognosis for the mother is obtained. Krishna Menon, for instance demonstrated in his comprehensive Indian series that all the various types of treatment employed prior to the "lytic cocktail resulted in a maternal mortality of about 15 per cent, while during recent years the mortality has been only slightly above 2 per cent.

Case Material

During the years from 1957 to 1964 all patients with toxaemia were consistently treated according to the following principles

1 The majority of the patients were booked cases with satisfactory antenatal care
2 The diagnosis was uniform, following the criteria of Trolle.
3 Cases which were diagnosed before the 36th week, were admitted at once to the antenatal ward for clinical observation and treatment by bed rest, diuretics. In recent years regular determinations of the urinary excretion of oestriol were carried out Labour was sometimes induced before the 36th week if indicated on clinical grounds, but in general efforts were made to continue the pregnancy until the 36th week. After this time irrespective of the condition of the patient, labour was always induced 3 or 4 weeks before term.

4 Cases which were diagnosed after the 36th week were admitted immediately for induction of labour In some cases a few days of conservative treatment before induction was considered to be beneficial

5 Cases of eclampsia were treated with

A. Intravenous infusion of 500 ml 5 % glucose containing 2 5 mg of Reserpinum NFN 50 mg of dihydralazini methansulfonas and 25 mg of chlorpromazini chloridum NFN The initial rate of infusion was 4–6 drops per minute The aim was to maintain a mean blood pressure of about 100 to 115 mm Hg, *i e* corresponding to a systolic pressure of about 150 mm Hg Because of the danger of hypoxia in the fœtus and shock in the mother the pressure was not allowed to fall to levels significantly below these values.

B If labour had not started or if there were slight contractions only an intravenous infusion of 5 I U of syntocinon *and 40 mg of papaverin in 500 ml 5% glucose was started* simultaneously at a rate of about 4 to 6 drops per minute

If the convulsions continued in spite of treatment or if delivery did not occur within a reasonable time Cæsarean section was performed

Results

The material comprises 26,400 deliveries with a total of 1,386 cases of toxæmia Table I presents the classification into groups the frequency of prematurity within the groups and the perinatal mortality The incidence of toxæmia corresponds roughly to standard. The distribution between slight and severe cases and cases of eclampsia shows that the frequency of eclampsia in the present series is minimal

The maternal mortality was *nil*.

In patients with eclampsia Cæsarean section was necessary in 3 cases (13 per cent)

With a view to the perinatal mortality rate the material is classified into two groups. One group comprises all patients who went into labour spontaneously while the other group comprises

Table I. Number of Cases of Toxemia Distributed into Diagnoses and the Corresponding Frequency of Prematurity and Perinatal Mortality

	Mild	Severe	Eclampsia	Total
Number	1 242	2	23	386
per ,000 deliveries	47	4 6	0 9	52 5
2,500 g	1 5	46	44	15
Perinatal mortality-rat per ,000 deliveries	3	207	130	40

$$\frac{\text{Toxemia mild + severe}}{\text{eclampsia}} = \frac{1,363}{23} = 59$$

Table II. Number of Cases of Toxemia Distributed According to Mode of Delivery and Incidence of Prematurity

Toxemia	Spontaneous Delivery	Induced Labour	Total
Mild	632	6	,24
Severe	5	70	1
Eclampsia	4	8	22
Total	697	658	385
infant 2,550 g	7	3	5

Table III. Perinatal Mortality Rat per ,000 Cases Perinatal Mortality Rates Classified According to Diagnoses and Mode of Delivery and Subdivided into Premature and Mature Cases

Toxemia	Total Spont /Induced	500 Spont /Induced	2 500 g Spont /Induced
Mild	4 — 1	9—	74— 0
Severe	204— 43	3—26	583—291
Eclampsia	4—	43—	429—
Total	3—	—3	286— 4

the patients in whom medical induction was performed, following conservative treatment where indicated.

It appears from Table II that the two groups were approximately the same size but that the frequency of prematurity was somewhat higher among those in whom spontaneous labour occurred than among those who had medical induction of labour

of here were admitt d with convulsions while 3 developed convulsions during bour ury in the antenatal ward

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The perinatal mortality rate which is by definition the number of deaths before during and one week after delivery per 1 000 cases is detailed in Table III

Discussion

The final calculations show that the results are extremely satisfactory as regards the infants and that the danger to the mother is negligible

Of course the groups of spontaneous deliveries and medical inductions cannot be compared directly because the spontaneous group includes several very small infants and also a number of cases of intrauterine death of the foetus occurring before toxaemia was diagnosed.

There were 18 infants delivered spontaneously and weighing under 1 500 g and 7 below this weight delivered following induced labour If the mortality rate for all cases over 1 500 g is calculated separately it appears that the perinatal mortality rate in spontaneous deliveries is 43 as against 9 in medical inductions These results show that active treatment with induction of labour at about the 36th week in all cases where induction is not necessary at an earlier time and immediate induction of labour in all cases diagnosed after the 36th week, does not involve any further danger to the infants but in all probability quite the opposite

SUMMARY

Over the years from 1957 to 1964 1 386 cases of toxaemia were encountered in 26 400 deliveries. The frequency of mild pre-eclampsia was 47 per 1 000 deliveries of severe pre-eclampsia 5 per 1 000 deliveries and of eclampsia 0 9 per 1 000 deliveries. The ratio between pre-eclampsia and eclampsia was 59 1 The overall perinatal mortality rate was 40 per 1,000

The value of induction of labour 3 or 4 weeks before term is discussed.

The maternal mortality was *nil*

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The perinatal mortality rate which is by definition the number of deaths before during and one week after delivery per 1,000 cases is detailed in Table III

Discussion

The final calculations show that the results are extremely satisfactory as regards the infants and that the danger to the mother is negligible

Of course the groups of spontaneous deliveries and medical inductions cannot be compared directly because the spontaneous group includes several very small infants and also a number of cases of intrauterine death of the foetus occurring before toxaemia was diagnosed

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The value of induction of labour 3 or 4 weeks before term is discussed.

The maternal mortality was *nil*.

Table I. *Sources and Nature of Malignant Trophoblastic Tumours Investigated*

	Chorionepithelioma Malignum	Destructive Mole	Unclassified
Admitted to The Norwegian Radium Hospital 1953–1961	35	5	
Admitted to other hospitals 1953–1961	1	4	1
Total	36	9	3

Table II. *Hydatidiform Mole, Destructive Mole and Chorionepithelioma in Norway 1953–61*

Year	Hydatidiform Mole	Destructive Mole	Chorionepithelioma Malignum
1953	26		3
1954	35		4
1955	48	1	
1956	45		
1957	56		
1958	57		4
1959	42		3
1960	49	1	4
1961	47	0	
Total	405	6	

hospital (J. Efskind, M.D.) without his knowing the ultimate fate of the patients. The pathological criteria used were the same as those of Novak and Seah (1954).

We have also received information from all the pathological institutes in Norway about the benign hydatidiform moles diagnosed during the years of compulsory cancer registration 1953–61. Presumably extremely few hydatidiform moles are not examined histologically today, so that the number of benign moles as reported by the above-mentioned institutes should give a fairly correct estimate of the yearly number of these tumours in the country.

The composition of the series of malignant trophoblastic tumours is presented in Table 1. In the three unclassified cases histological examination of endometrial curettings showed the

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TROPHOBLASTIC TUMOURS IN NORWAY

BY

PER KOLSTAD[1] AND JENS HOGNESTAD

The incidence of chorionepithelioma malignum destructive mole and benign hydatidiform mole varies considerably in different parts of the world, but is much greater in the Orient than elsewhere (Acosta-Sison 1949 *Joint project* 1958 Novak and Woodruff 1962) No reports have appeared on the occurrence of such tumours in Norway since Sunde in 1920 published his series of chorionepithelioma malignum From 1952 however there has been compulsory registration of all malignant diseases in Norway and consequently today it is possible to give the exact number of malignant trophoblastic tumours diagnosed each year within this geographically defined area. The aim of the present paper is to contribute to our knowledge of the incidence and the prognosis of chorionepithelioma malignum and destructive mole and of the relationship between these tumours and benign hydatidiform mole

Series

Our material consists of 32 cases of malignant trophoblastic tumours treated in The Norwegian Radium Hospital during the 30-years period 1932–61 and of 16 cases from other Norwegian hospitals reported to the Norwegian Cancer Registry during the years 1953–61 The pathological diagnosis has been revised in 37 of the total number of 48 cases by the senior pathologist at our

[1] Fellow of the Norwegian Cancer Society

Table IV *Incidence of Malignant Trophoblastic Tumours According to Parity*

Parity	Destructive Mole	Chorionepithelioma Malignum	Total
0		8	9
I		1	13
II			3
III		8	8
IV			3
V or more	5	4	9
Total	9	36	45

Table V Nature of Preceding Pregnancy

Preceding Pregnancy	Destructive Mole	Chorionepithelioma Malignum	Total No.	Total Per cent
Hydatidiform mole	6		6	35 6
Abortion		12	14	3 .1
Term pregnancy			12	26 6
Unknown			3	6.7
Total	9	36	45	100 %

other hand, 20 per cent had delivered five or more children an exceptionally high proportion when compared with the normal parity distribution in the population.

The relationship between the malignant trophoblastic tumours and the preceding pregnancy is shown in Table V A little more than one third of the tumours developed after hydatidiform mole about one third after abortion and less than one third after term pregnancy In two cases of destructive mole the prior gestation terminated in abortion. A more detailed study of the gestation products in abortions might have revealed a higher incidence of hydatidiform degeneration as demonstrated by Hertig and Edmonds (1940) and by Nilsson (1957)

The time interval between the preceding pregnancy and the diagnosis of a malignant trophoblastic tumour is presented in Table VI All the 9 cases of destructive mole were diagnosed within six months For chorionepithelioma a longer time interval elapsed before the disease was discovered, although as many as 27 of the 36 cases (75 per cent) were diagnosed within six

Table III. *Age Distribution in 45 Cases of Malignant Trophoblastic Tumours*

Age	Destructive Mole	Chorionepithelioma Malignum	Total
15–19	0	1	1
20–24	1	3	4
25 29	1	10	11
30–34	1	6	-
35–39	1	8	9
40–44	3	3	6
45–49	2	2	4
50–54	0	3	3
Total	9	36	45

possibility of chorionepithelioma but when hysterectomy was performed histological examination of the operation specimen revealed no signs of tumour All three patients are still alive In the following tables these three cases are excluded.

For the years 1953–61 it is possible to give an estimate of the incidence of the different types of trophoblastic tumours in Norway (Table II) An average of 45 benign moles and 3 malignant trophoblastic tumours was diagnosed each year The annual number of births (over 28 weeks and including stillbirths) was approximately 60 000 for the period covered by the Table Thus, the frequency of vesicular mole was approximately 1 1 300 deliveries and that of malignant trophoblastic tumour 1:20 000 deliveries Of the total of 405 hydatidiform moles 12 became malignant (2 96 per cent) Chorionepithelioma developed in 9 cases (2 22 per cent) and destructive mole in 3 cases (0 74 per cent)

The age distribution of the 45 patients with malignant trophoblastic tumours is shown in Table III One patient was 19 years old when a diagnosis of chorionepithelioma malignum was made and three patients were in the age group 50–54 years. In 60 per cent of the cases the patients were between 25 and 39 years of age

In Table IV the incidence according to parity is presented. It is remarkable that in about 50 per cent of the patients the tumour developed during their first or second pregnancy On the

Table VII. Follow-up Results of Destructive Mole and Chorionepithelioma Malignum

Time Interval between Diagnosis and Death	Destructive Mole	Chorionepithelioma Malignum
– 2 months	0	7
3– 6	0	14
7–		4
13– 8	0	1
Total number dead	0	26
Total number alive*	9	10
Survival rate*	100 %	27.8 %

All patients observed more than years.

The follow-up results are shown in Table VII, which demonstrates an entirely different prognosis in the two groups of malignant trophoblastic tumours. All of the 9 patients with destructive mole are living and well, 8 of them for more than five years. However only 10 of the 36 patients with chorionepithelioma survived (27.8 per cent) It is of great interest to note that one of these patients had metastases in the lungs at the initial examination. After hysterectomy there was a spontaneous regression of the lung metastases. She has now been observed for 11 years without any signs of recurrence The pathological specimen has been reviewed several times but different pathologists agree upon a diagnosis of chorionepithelioma malignum.

Three patients had chorionepithelioma with metastases in the vagina and removal of the metastases and hysterectomy were performed. All three patients are living and well with an observation time of 2 13 and 22 years respectively

Discussion

As mentioned above both benign and malignant trophoblastic tumours are much more common in the Far East. The reason for this is unknown. The incidence of benign hydatidiform mole in Norway of 1 1 300 deliveries may be compared with reports from other Western countries. Chesley Cosgrove and Preece (1946) found 1 in 1,321 deliveries Haines and

Table VI. *Time Interval between End of Preceding Pregnancy and Diagnosis of a Trophoblastic Tumour*

Time Interval	Destructive Mole	Chorionepithelioma Malignum	Total No	Total Per cent
– 2 months	8	12	20	44.4
2– 6 "	1	15	16	35.6
7–12 "	0	3	3	6.7
1– 2 years	0	2	2	4.4
2– 4 "	0	2	2	4.4
Unknown	0	2	2	4.4
Total	9	36	45	100 %

months Two of our patients had an unusually long symptom free period between the gestation and the development of chorionepithelioma One of these a 37 years old woman, was admitted to the hospital because of intestinal bleeding appearing three years after she had delivered a hydatidiform mole. Chest X ray disclosed multiple lung metastases The patient died five months later and autopsy showed chorionepithelioma in the pelvis intestines and lungs. Another patient had en abortion two years before she was found to have a tumour in the vulva. Removal of the tumour and hysterectomy was performed. Histological examination showed chorionepithelioma in both sites. Soon afterwards she developed brain metastases and died. There was no history of menstrual irregularity or bleeding in the symptom free interval in these two patients

Routine treatment for our patients has been hysterectomy with bilateral salpingo-oophorectomy Big polycystic ovaries probably caused by the excessive amount of chorionic gonadotrophins in the serum were observed in 8 cases (17 8 per cent) but none of the patients showed ovarian metastases In addition radium and deep X ray treatment was given in some cases with apparently little or no effect except for one case where vaginal metastases disappeared after radium applications. Methotrexate was used in four cases with good initial response in one case Our experience with this drug, however is limited. Publications from other centres indicate that methotrexate today is the drug of choice in the treatment of chorionepithelioma (Hertz et al 1958 1961)

Novak and Seah (1954) In the present study all the patients with destructive mole survived, while the survival rate for the patients with chorionepithelioma was only 27.8 per cent. It should also be mentioned that the prognosis was better in cases developing after a mole This may at least in part be explained by the routine use of pregnancy tests in the follow-up of patients with hydatidiform mole Thereby an earlier diagnosis of malig nancy was made in these cases as compared with tumours de veloping after abortion or term pregnancy (cf Delfs, 1957) The patients with chorionepithelioma who died did so within 1½ years following the diagnosis, most of them within six months.

Chorionepithelioma malignum is peculiar in that regression and spontaneous cure may be noted not only in the primary tumour but also in metastases (Novak and Koff 1930 Park and Lees, 1950) We are able to add a new case of spontaneous disappearance of lung metastases after removal of the primary tumour in the uterus to the relatively long list of such cases in the medical literature

SUMMARY

A series of 405 benign hydatidiform moles 36 chorionepi theliomas and 9 destructive moles is presented. An average of 45 benign moles and 3 malignant trophoblastic tumours are diag nosed each year in Norway where compulsory cancer registration was established in 1952. The frequency of vesicular mole was calculated as approximately 1 1 300 deliveries, and that of malig nant trophoblastic tumours as 1 20,000 deliveries. Of the 405 moles chorionepithelioma developed in 9 cases (2.22 per cent) and destructive mole in 3 cases (0 74 per cent) The malignant trophoblastic tumours were preceded by a mole in 35.6 *per cent*, by abortion in 31 1 per cent, and by term pregnancy in 26 6 per cent. In 6 7 per cent of the series the history of the preceding pregnancy was uncertain

All of the 9 patients with destructive mole are living and well, 8 of them for more than five years. Only 27.8 per cent of the patients with chorionepithelioma malignum survived. In one patient with multiple lung metastases on the initial examination

Taylor (1962) mention figures between 1 1,000 and 1:2 000 deliveries and Novak and Woodruff (1962) give an average of about 1 in every 2 500 pregnancies.

Both Haines and Taylor (1962) and Novak and Woodruff (1962) estimate the proportion of hydatidiform moles which become malignant at from 1 to 2 per cent. In the present work a figure of 2 96 per cent was found, (0 74 per cent destructive mole and 2 22 per cent chorionepithelioma)

In 35 6 per cent of the cases the malignant trophoblastic tumours were preceded by hydatidiform mole 31 1 per cent by abortion and 26 6 per cent by term pregnancy In 6 7 per cent of the cases the preceding pregnancy was uncertain Sunde (1920) in his series from the same geographic area found a preceding pregnancy to be mole in 55.2 per cent, abortion in 21 1 per cent, term pregnancy in 21 1 per cent and he also reported a case which developed after an extrauterine pregnancy The figures in these two series from Norway do not differ greatly from those published from other Western countries (Haines and Taylor 1962 Novak and Woodruff 1962)

The problem of the so-called long latency is a debatable phenomenon in the behaviour of malignant trophoblastic tumours (Park and Lees 1950) As long ago as 1920 Sunde observed that there may be a long period without symptoms between parturition or miscarriage and the development of chorionepithelioma In the present study two patients showed a long period of latency two and three years respectively One must of course always consider the possibility that an unsuspected abortion has intervened in such cases This seems however not to have occurred in our two patients Novak and Woodruff (1962) describe a chorionepithelioma which evolved nearly four years after a normal delivery at which time tubal ligation was carried out because of hypertension A long period of latency must be accepted as a possibility when a diagnosis of malignant trophoblastic tumour is made

To distinguish between destructive mole and chorionepithelioma is of utmost importance as the prognosis is entirely different in these two groups Some of the hazards in making a diagnosis of malignant trophoblastic tumour was clearly demonstrated by

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THE DEVELOPMENT OF HYPOPHYSEAL, OVARIAN AND MAMMARY GLAND TISSUES GRAFTED SIMULTANEOUSLY TO THE ANTERIOR CHAMBER OF THE EYE OR THE SPLEEN OF GONADECTOMIZED RATS

BY

STIG KULLANDER

In an earlier publication (1960) the present author showed that when small pieces of ovarian and hypophyseal tissues were grafted simultaneously to the anterior chamber of the eye of two-month old spayed rats they developed into tumours—mixed granulosa cell tumours and chromophobic hypophyseal adenomas. These tumours showed signs of oestrogen-progesterone and prolactin secretion respectively. There was a remarkable development and stimulation of the mammary glands in the hosts.

This work now has been extended. Some of the operations have been made on gonadectomized male rats to compare the sensitivity of their mammary glands with the glands of gonadectomized female hosts. In an attempt to ascertain whether the hormones produced by the mixed ovarian-hypophyseal tumours could give rise to mammary stimulation or tumours by local action, fragments of mammary gland tissue were grafted to the anterior chamber of the eye in addition to the ovarian and hypophyseal tissue. All these types of tissues were also transplanted simultaneously into the spleen of some gonadectomized male and female rats and their subsequent development studied.

Material and Method

The animals were two months old at operation and belonged to the homozygous R-strain. The incidence of spontaneous mammary tumours in this strain is very low. The rats were kept

spontaneous regression of the metastases occurred after removal of the primary tumour The observation time for this patient is now 11 years.

In two patients a long period of latency between the preceding pregnancy and the diagnosis of the malignant trophoblastic tumour was observed, more than two and more than three years respectively

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Fig. Corpora lutea surrounded by hypophyseal tissue. In the upper right corner splenic tissue can be seen. Two months after grafting ovarian + hypophyseal + mammary gland tissue to spleen of gonadectomized rat. 5 section Hematoxylin-eosin staining. Magnification, 200 ×

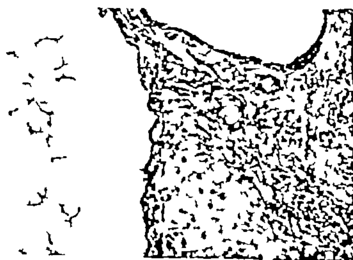


Fig. Compare Figure. Mammary gland tissue growing next to corpus luteum. section Hematoxylin-eosin staining. Magnification 200 ×

Table I *Type of Host and Donor Tissue*

Type of Transplantation	No. of Animals
Piece of ovary hypophyseal anterior lobe and mammary gland into spleen of gonadectomized animals	8 ♂ + 4 ♀
Piece of ovarian + piece of hypophyseal anterior lobe and mammary gland into rigth eye piece of mammary gland into left eye of gonadectomized animals	6 ♂ + 7 ♀
Total	*14* ♂ + *11* ♀

four or five to a cage and given commercial pellets and tap water *ad libitum* Bilateral gonadectomy was performed under ether anæsthesia through a midline abdominal incision A piece of ovarian tissue about as large as a medium-sized follicle was grafted to the anterior chamber of the right eye (dissection and grafting under ×15 magnification) of gonadectomized hosts together with a small piece of an anterior hypophyseal lobe (from two-month old male rats) and of mammary gland tissue (from a three-month old lactating rat) For control purposes mammary gland tissue was grafted to the anterior chamber of the left eye In some gonadectomized animals the tissues were grafted to the spleen The technique of grafting to the spleen was that previously described by the present author (1956) for the induction of ovarian tumours After operation the hosts were killed at various ages ranging from 2 months to 2 years The grafts were removed, fixed in Susa embedded in paraffin and sectioned. The animals were carefully autopsied, special studies being made of the development of the mammary glands. Enlarged or tumourous mammary glands were removed, fixed, embedded and sectioned in the same way as the grafts All sections were stained with hæmatoxylin-eosin The types and numbers of transplantations are shown in Table I

Results

Ovarian + hypophyseal + mammary gland transplants to the

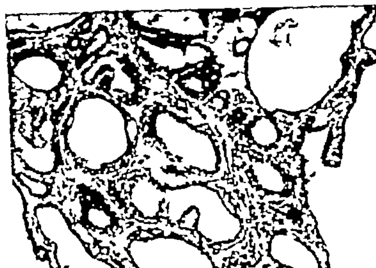


Fig 4 Mammary adenoma growing subcutaneously in an intact female rat 4 months after grafting mammary tumour (Fig 3) induced in the spleen. 5 section Haematoxylin-eosin staining Magnification. 200 ×

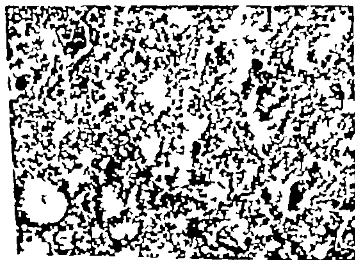


Fig 5 This mammary tumour developed years after grafting into the spleen of gonadectomized male rat. 5 section Haematoxylin-eosin staining Magnification. 200 ×

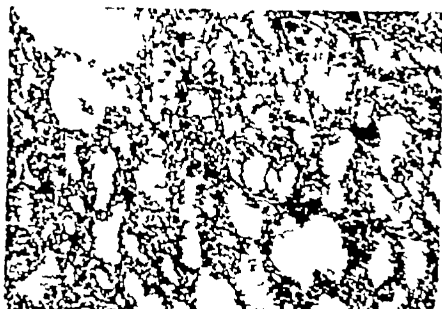


Fig. 3. One year after grafting ovarian + hypophyseal + mammary gland tissue to spleen of a gonadectomized rat. Secreting mammary adenoma in spleen. 5 section Hematoxylin-eosin staining. Magnification. 200 ×

to proliferating growths of various sizes. Microscopic investigations revealed large well-developed corpora lutea formed by the ovarian tissue and a uniform mat of chromophobic cells formed by cells from the hypophyseal tissue (Fig. 1). There was very little growth of the mammary gland tissue but signs of secretion were present (Fig. 2). In animals killed 4, 6 or 9 months after operation the grafts had undergone histological changes, appeared tumorous in some instances, and were much larger. At 9 months there was a granulosa-cell tumour and a chromophobic hypophyseal adenoma. The mammary gland tissue exhibited *moderate proliferation but showed signs of secretion.* One year after transplantation, however, grafted mammary gland tissue had formed secreting mammary adenomas (Fig. 3). When such a mammary adenoma from the spleen was transplanted subcutaneously to an intact female rat it grew progressively. Four months after the grafting a biopsy from this subcutaneous growth revealed a papillomatous mammary adenoma without secretion (Fig. 4).



Fig 7 Two months after grafting to a gonadectomized male host. Corpus luteum with signs of intense activity 5 section Haematoxylin-eosin staining. Magnification 700 ×

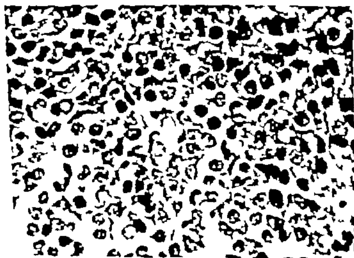


Fig 8 Hypophyseal chromophobic tumor in the anterior chamber of the eye Two month after grafting Gonadectomized male host 5 section Haematoxylin-eosin staining Magnification 700 ×

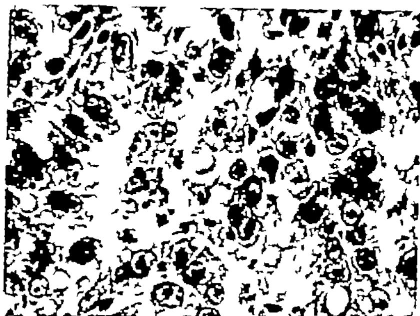


Fig 6 Compare Figure 5 5μ section Hematoxylin-eosin staining Magnification 700 ×

At this time the host mammary glands *in situ* were developed to some extent and contained some milk but no tumours were observed.

When animals were killed two years after grafting to the spleen (1 ♂ and 4 ♀) the spleen contained mammary tumours in addition to granulosa cell tumours and chromophobic hypophyseal adenomas Microscopically the mammary tumours exhibited wild, abnormal hyperplasia with absence of all normal structures (Figs 5 and 6) They had a tendency to papillomatous growth. In some solid parts with sparse secretion there was polymorphism *and abnormal mitosis evidence of early malignant growth. In all* these animals the mammary glands *in situ* were highly stimulated. In one of them (a spayed female) a mammary tumour (40 × 30 × 10 mm) *in situ* had developed. Histologically it was an early cancer with polymorphism and abnormal mitoses

Ovarian + hypophyseal + mammary gland transplants to the anterior chamber of the eye These grafts also took and grew progressively ultimately being transformed into tumours bulging and destroying the eye The single piece of mammary gland tissue



Fig. 10 Mammary tumour *in situ* in regression, in male gonadectomized rat. Four months after enucleation of eye with hypophyseal-ovarian tumour 5 section Hematoxylin-eosin staining. Magnification 700 ×



Fig. Mammary gland *in situ* (pre-tumour) in spayed rat one year after spaying and grafting of ovarian + hypophyseal + mammary gland tissue to the anterior chamber of the eye 5 section Hematoxylin-eosin staining Magnification 700



Fig. 9. Compare Figure 8. The mammary gland graft. 5μ section Hæmatoxylin-eosin staining. Magnification 200 ×

in the other eye took but made very little growth. Two months after operation follicles and well-developed seemingly highly active corpora lutea (Fig 7) were formed by the ovarian tissue the hypophysis showed a uniform mass of chromophobic cells (Fig 8) and the mammary gland tissue showed signs of secretion (Fig 9) In animals killed after 4 6 or 9 months there was a gradual shift to granulosa-cell tumour and chromophobic hypophyseal adenoma the mammary gland tissue had grown slowly but showed signs of secretory activity

One year after grafting (1 ♂ and 5 ♀) the eyes with multiple grafts were ulcerated and filled with tumorous infected masses making detailed histological examination difficult. The mammary glands in situ in all these animals were highly stimulated and secreting as were though to a lesser degree the single mammary gland grafts in the chamber of the left eye

In the gonadectomized male host, two mammary tumours were observed growing in different mammary glands *in situ* They measured 40×30×10 mm. and 10×10×5 mm. respectively When the eyes were enucleated these mammary tumours *in situ*

tissue is ultimately formed because of the marked stimulation. The sustained growth of such mammary tumours is probably dependent on continuous hormonal stimulation. This idea is supported by the finding that mammary tumours *in situ* regressed after enucleation of the eye. However extirpation of the hypophyseal-ovarian tumour in the eye may still leave some prolactin produced from the hypophysis *in situ* and steroids from the adrenals. The histological picture in the regressed mammary tumours 4 months after the enucleation of the eye was still somewhat abnormal.

Mammary gland grafted by itself to the anterior chamber certainly reacted to the hormonal stimuli but apparently to a somewhat lesser degree than the glands *in situ*.

Mixed grafting to the spleen seems superior to mixed grafting to the chamber of the eye for studies of the local influence of prolactin and steroids on the mammary glands. There is insufficient room in the chamber of the eye for all the tumours formed the eye is easily destroyed, ulcerated and infected.

The spleen is excellent for long-term studies of tumour development in those mixed grafts. As early as 2 months after grafting to the spleen there was milk-secretion in the mammary gland grafts but not in the mammary glands *in situ*. At the same time there were well-developed corpora lutea in the ovarian grafts. Progesterone from the splenic-grafted ovarian tissue and prolactin from the grafted pituitary may be the locally acting stimuli for this milk-secretion. It does not seem necessary for progesterone and prolactin to be first metabolized before acting upon the mammary gland target organ.

This would be in accord with observations made in organ tissue cultures of whole mouse mammary glands. Adding prolactin and progesterone to the culture medium stimulates growth and secretion of the mammary gland (Prop 1960)

Mammary tumours may also be induced by local action alone However steroids, mainly progesterone (Kullander 1956) from the ovarian graft or tumour pass, to some extent, through the liver and into the general circulation without being inactivated. They then act upon all the mammary glands *in situ* as does prolactin from the pituitary graft, resulting in growth milk

regressed quickly and almost completely Four months after the enucleation of the eyes a biopsy from the now just palpable masses showed a "silent", but not normal picture (Fig 10) with wide ducts and quiet epithelium. In three out of five spayed female hosts examined small white firm nodules up to the size of a pea were seen in the mammary glands *in situ* Histologically (Fig 11) they showed a hyperplastic precancerous picture with thick abnormal epithelium. No rat in this group was followed to an age greater than one year They all had to be killed due to their eyes with infected ulcerated tumours.

Discussion

Prolactin and prolactin producing hypophyseal transplants or tumours are probably related to mammary tumour genesis besides progesterone and oestrogen Thus has been shown in rather extensive studies in mice (see Mühlbock and Boot, 1959 Boot *et al* 1962 and others) and probably also occurs in rats (see Dao and Gawlah 1963 Kullander 1960 and others) The present experiments confirm and extend those findings as far as rats are concerned.

Continuous increased prolactin production is certainly present in the animals with hypophyseal-ovarian grafts in the anterior chamber of the eye A transplanted pituitary produces prolactin almost exclusively (see Boot *et al.* 1962) This function is probably accentuated when the pituitary forms a chromophobic tumour There is in addition a supply of steroid hormones from the induced granulosa-cell tumours in the grafted hosts. The induction of ovarian tumours in the chamber of the eye is due to the disturbed hormonal balance with overproduction of gonadotrophic hormones following gonadectomy A small fragment of ovarian tissue does not produce enough steroids to counteract this. Implantation of small ovarian fragments into the chamber of the eye of an intact female does not give rise to tumours (Kullander 1961) Milk secretion and growth is induced in the mammary gland grafts in the chamber of the eye as well as in the *mammary glands in situ* This has been observed in male and female gonadectomized hosts Precancerous or cancerous

hypophyseal tumour can be made at different stages of tumour development in mammary gland tissue transplanted or *in situ*

Nothing in the present experiments indicated a difference between male and female rats in sensitivity or response of the mammary glands to the type of endocrine set-up used.

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secretion and ultimately tumour formation of the glands *in situ*. Prolactin passes through the liver without being inactivated (see Boot *et al.* 1962) It is probable that some prolactin is produced in the hypophysis *in situ* also in these animals. This is indicated by the fact that luteinized ovarian tissue in the spleen of spayed rats produces progesterone (Kullander 1956)

More detailed and extensive experiments will have to be carried out with regard to a) the possibilities of transplanting the mammary tumours induced in the spleen or *in situ* and b) their hormone-dependency at different stages of development.

The mammary gland grafts grew and were stimulated in the spleen when situated at some distance from the ovarian and pituitary grafts. This indicates diffusion within the splenic parenchyma of the ovarian and pituitary hormones produced and is in accord—as far as steroids are concerned—with the findings of Gitsch (1958) It is then possible to take biopsies from or make complete excision of various tumours at different tumour ages.

Nothing in the present experiments indicated a difference between male and female rats in sensitivity or response of the mammary glands to the type of endocrine set up used

SUMMARY

When small pieces of hypophyseal ovarian and mammary gland tissue are simultaneously transplanted to the anterior chamber of the eye or to the spleen of gonadectomized rats tumours may form in all the grafted tissues Hormones from the ovarian and hypophyseal grafts or tumours (granulosa cell tumour and chromophobic hypophyseal adenoma respectively) act locally on the mammary gland transplant but also pass into the general circulation giving rise to hyperstimulation and sometimes tumour formation in the mammary glands *in situ*

In the spleen the tissues may be transplanted at some distance from each other making it possible to watch the growth of the different tumours formed and to obtain biopsy specimens during their life-span

With these types of experimental set-up complete excision of the steroid-producing ovarian tumour and/or prolactin secreting



Fig 1

There is another reason for these modifications. I am not convinced that the V E. should replace the forceps in cases of foetal distress when the fœtal head is on the pelvic floor But I think that the V E. should substitute the forceps when the head is higher up in the pelvis with the sagittal suture in the transverse or oblique diameters. I have previously tried a modified scalp forceps and also the application of a girdle around the foetal head above the biggest circumference to achieve a grip for prolonged traction. The appliances were however not ideal. For this reason I have tried to modify the suction cup for use in prolonged labour at a stage where forceps should not be used.

To apply a suction cup between the occiput and the pelvic wall with the head in the transverse diameter the cup must be thinner in that part which reaches highest up in the pelvis.

The second difference from Malmström s cup is the attachment of the suction mechanism and the traction chain. In Malmström s cup both are in the centre Assymetric traction will then result in a stronger pull on the side of the cup turned away from the line of traction and this will increase the risk of slipping.

To prevent the cup from turning over by side-traction, I made

Lövset J., Acta obst. et gynec. scandinav. 44, 102, 965

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MODERN TECHNIQUES OF VAGINAL OPERATIVE DELIVERY IN CEPHALIC PRESENTATION

BY

JÖRGEN LÖVSET

Since the obstetrical forceps was invented round about 1518 it has been *the* instrument for delivering the head in cephalic presentation. The attempts made from the time of John Yonge (1706) to use some form of suction instrument met with no real success until recently Several new instruments of this kind have been invented in our time Some of them have found a place in clinical practice for example Couzigous (1947) ventouse eutocique in France Kollers (1950) instrument in Norway and some others. None of these has achieved the same degree of success as Malmströms (1954) Vacuum Extractor (V E.) Since its introduction the instrument has been further developed. The vacuum which in the first instance was produced manually is now induced by a vacuum motor as proposed and used by Evelbauer (1956)

The construction of Malmströms V E. makes it mandatory to use a line of traction approximately at right angles to the cup. If traction is applied at an acute angle the cup tends to slip off Lange (1964) comments on this slipping of the cup in his cases in these words. *but most commonly an incorrect line of* traction is the reason why the cup slips Out of a total of 895 deliveries by V E *the cup slipped in 149 cases*

The implication of the detachment is difficult to assess but it is, to say the least, an inconvenience This is the reason why I have tried to minimize the risk of slipping by altering the construction of the cup and especially the traction attachments.

anterior axilla. Lastly the forceps should be used to protect the head in prematurity

The V E. has given us the opportunity to help in situations where the forceps should not be used, and there is no question about its great value It is natural that it will be tried and used, to begin with, in cases where the forceps might be better As we gain experience in the future a proper place will be assigned to each of them.

Another competitor to the forceps was announced last year by P Radvic (1963) from Belgrade He introduced what he called forceps pharmaco-dynamique (F F) The method consists of giving Syntocinon 2–3 I U in 20–50 ml 50 %Glucose. This solution is given intravenously very slowly till the first contraction starts. Then the injection is interrupted to wait for the next contraction. If it does not come reasonably quickly the injection is continued.

This method is used in primigravidae with the foetal head on the pelvic floor and the sagittal suture in antero-posterior diameter

In multiparae it may be tried even if internal rotation is not complete Then the patient has to be turned over to the side on which the foetal occiput lies, when the injection starts. It is necessary to use 3–5 I U Syntocinon in 30–50 ml 50 % Glucose in these cases.

The indications for using F F are weak pains or rigidity of the soft parts causing delay in the second stage. It may also be used in foetal distress and in breech delivery F F in breech delivery requires 5 I U Syntocinon in 20 ml 50 % Glucose. Contraindications are

1 Deflexion

2 When internal rotation has not begun in primigravidae and

3 Occipito posterior position.

The duration of the delivery of the head should not be more than 2 3 minutes If it lasts longer the delivery can not be finished by this method and one must resort to forceps or V E.

Last year there was published another method which is somewhat similar to the F F (C Revaz, 1963) It may not be called a competitor to the forceps because it is introduced to reduce

four holes for fastening nooses on two loops of cord. To each of these loops a block is attached and from each of the blocks goes a third loop with a third block. This arrangement will distribute the traction evenly around the periphery of the suction cup and the tendency to slip off when side traction is applied, is minimized.

At 45° the cup withstands a side-traction of 9 kg with a negative pressure of 0.5 kg per cm² while the same size of Malmström's cup withstands 3.7 kg with side-traction at 45° and at the same negative pressure.

Apart from the risk of slipping off with side-traction it is not possible to apply Malmström's V.E. in the ideal position when the sagittal suture is in the transverse diameter. Evelbauer, Hathout and Tannir (1963) speak about auto-rotation which means that the head rotates correctly even if the V.E. is applied over the parietal bones. That is probably correct. But in operative obstetrics we should lay stress on *aiding* the expelling *forces and the correct rotation.*

Personally, I do not like to replace the forceps by a V.E. in foetal distress even if it is possible to establish a high and constant vacuum in a few seconds with an electrical pump. Especially where trained anesthetists are available. I would also prefer the forceps for mid-cavity extraction in cases of foetal distress if I knew that the foetal head moved down to the pelvic floor during pains. The reason for my preference is that the correctly applied forceps never slips. Perhaps V.E. is preferable for the tyro because application of the forceps requires a correct diagnosis and a biparietal grip.

With occipito-posterior position I would also prefer forceps, especially when we have forceps which can be applied without wandering like the one I showed on a congress at Munich in 1934 and published in Acta obst. et gynec. scandinav. in 1937. With a breech presentation there is no need for any instrument until the shoulders are delivered. Then the forceps is the best instrument with which to deliver the after-coming head if there is significant resistance.

In a difficult shoulder-delivery forceps is an excellent aid, not applied according to Shute (1962) but with the foetal head lifted and the forceps applied from the hind shoulder to the

von Numers, Claes, L., Acta obst. et gynec. scandinav. 44, 107 1965.

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OBSERVATIONS ON METAPLASTIC CHANGES IN THE GERMINAL EPITHELIUM OF THE OVARY AND ON THE ÆTIOLOGY OF OVARIAN ENDOMETRIOSIS

BY

CLAES VON NUMERS

The germinal epithelium of the ovary evidently possesses a considerable capacity for metaplasia as a reaction against stimuli of different kinds. This is shown by observations in clinical pathology as well as by information gained experimentally. However, disagreement still exists concerning the question: What different potencies can be ascribed to this epithelium and what is the possible aetiological significance of metaplastic changes for different pathological processes in the ovary?

The very frequent inclusion cysts occurring in the cortex of the ovary are, as indicated by their name, undoubtedly derived from the germinal epithelium. They consist of a single-layered, cubic or low columnar epithelium which often shows more or less marked metaplastic changes, generally towards a higher, partly ciliated type, resembling the epithelium of the Fallopian tube. These small inclusion cysts and the different changes in their epithelium form an interesting and valuable subject for investigation, particularly as their histogenesis is not in doubt.

Of the true tumours, the serous cystadenoma, according to the unanimous views of today, derives from the germinal epithelium. Concerning the pseudomucinous cystadenoma, on the contrary, opinion is not as uniform. According to many authors this tumour also originates in the germinal epithelium—an opinion which is supported among others by the fact that mixtures of serous and pseudomucinous cysts have been observed by Gault *et al.* (1954)

Caesarean section and either forceps or V E. can be resorted to if the operator is prepared to wait for suitable conditions.

The method has been used in Toulouse since 1959 and consists of giving Syntocinon together with Pentothal Syntocinon may be given intravenously in small doses 0 8 I U and Pentothal on an average of 0 37 gr This medication may start when the os is only 7 cm. It takes usually only a few minutes to deliver the foetus and it has never been known to take more than 10 minutes.

It is amazing that this method can be used also in foetal distress without harming the foetus.

As mentioned, the last two methods are very similar but the last one is much more radical as it can be used before the os is effaced. The reason for this may probably be that the delivery is terminated so rapidly that the foetus will not be seriously asphyxiated by the use of Syntocinon and the Pentothal will only reach it in minute doses

Many new techniques for aiding delivery of the foetus when the head is presenting have been developed during this century and further progress seems likely in the near future

SUMMARY

The main methods and means for delivering the head vaginally in cephalic presentation are given a short survey the forceps, the vacuum extractor forceps pharmocodynamique and the method developed in Toulouse since 1959 with Syntocinon and Pentothal are reviewed.

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Fig. Epithelial and stromal changes along the endometriosis line. Same case as Fig. Haem. + van Gieson. × 120. Slightly reduced



Fig. 3. Cyst formation with columnar epithelium of pseudomucinous type. Same case as Fig. and Fig. Haem. + van Gieson. × 20. Slightly reduced.



Fig. 1 Inclusion cysts with metaplastic epithelium, essentially of serous type. Haem. + van Gieson. × 120 Slightly reduced.

Fischer *et al* (1955) and Willis (1958) However other authors including Novak and Novak (1958) consider the pseudomucinous cystadenoma to be a teratoma which has differentiated along a single line In addition the fact that the Brenner tumour—which often occurs in combination with a pseudomucinous cystadenoma—is generally supposed to be of mesothelial origin seems to be against the latter conception

The problem of the genesis of endometriosis has attracted much interest. The metaplasia theory inaugurated by Robert Meyer and Sampson s theory of implantation have both gained numerous supporters The extensive relevant literature can not possibly be quoted in this connection I will only refer to Nicholsons (1926) and Willis (1958) reviews and cite the conclusions made by the last-mentioned author "It is difficult to understand how subsequent writers could have continued to prefer Sampson s implantation theory of peritoneal metaplasia at least for the great majority of cases of extra uterine endometriosis



Fig. 2. Epithelial and stromal changes along the endometriosis line. Same case as Fig. Haem. + van Gieson × 20. Slightly reduced.



Fig. 3. Cyst formation with columnar epithelium of pseudomucinous type. Same case as Fig. and Fig. Haem. + van Gieson. × 120. Slightly reduced.



Fig 4. Small cyst of endometrioid type. Ovarian surface covered by granulations caused by chronic oophoritis. Haem. + van Gieson. × 120. Slightly reduced.

As mentioned earlier the small inclusion cysts derived from the germinal epithelium constitute a valuable subject for histological examinations In the deep invaginations of the surface epithelium which are the precursors of the cysts as well as in the cysts completely separated from the surface the epithelium consists of a single row of columnar cells which are taller than those belonging to the surface epithelium proper (Figs 1 5, 6) At first the epithelium is of a more or less indifferent appearance but shows gradually increasing metaplasia and becomes partly ciliated. In places it can assume an appearance typical of the tubal epithelium or of the columnar epithelium in serous cystadenomas (Figs 1 5) in other places it may become reminiscent of the endometrium (Figs 2 4 6 7) Less frequently light stained columnar epithelium of the type occurring in pseudomucinous cysts is seen (Fig 3) However it must be admitted that to distinguish between these different types of epithelium in many

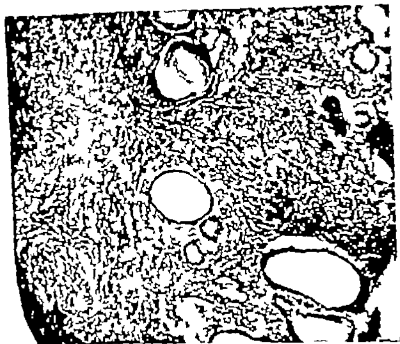


Fig. 5 Multiple inclusion cysts or adenofibroma? Same case as Fig. 4. Haem.+ van Gieson. × 120. Slightly reduced.

inclusion cysts especially those with incipient or otherwise low grade epithelial changes may be difficult or impossible. In such cases, where within a limited area of ovarian cortex several small cysts with epithelium of serous type are met with, it can often be problematic to decide whether multiple inclusion cysts are present, or whether there is a tumour of the adenofibroma type (Fig. 5) In some cases even small cysts reveal papillary excrescences typical of the papillary serous cystadenoma. This finding obviously points to a further differentiation towards the tumour type in question.

The stromal tissue surrounding the cysts with more or less typical serous epithelium consists partly of ordinary ovarian stroma partly of more dense fibrillary connective tissue poor in cells and of the same structure as that encountered in adenofibromas and in the wall of cystadenomas. In addition another

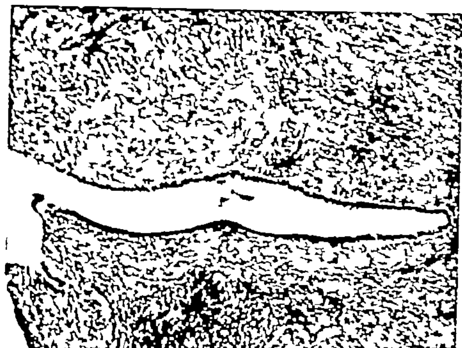


Fig. 6 Deep invagination of the surface epithelium. Commencing metaplastic changes of endometrioid type in epithelium and stroma. Haem.+van Gieson. × 120. Slightly reduced.

histologically important and interesting change of the juxta-epithelial stroma occurs. It consists of a loosening of the stromal tissue which simultaneously becomes comparatively richer in cells and poorer in fibrils assuming a reticular structure The further development of this results in a tissue completely corresponding morphologically to the stroma of the uterine endometrium or extrauterine endometriosis (Figs 2 4 6 7) The initial stage of this change can sometimes be observed beneath the surface epithelium proper however it is far more common in connection with the epithelium of invaginations or inclusion cysts which often reveals signs of metaplasia of indifferent type Examination of a sufficient number of suitable cases shows that the stromal change in question for the most part parallels the metaplastic process of the epithelium which results in columnar epithelium of endometrioid type. As a matter of fact all transitional stages can be observed from obvious inclusion cysts with scanty stromal changes to completely typical foci of endo-

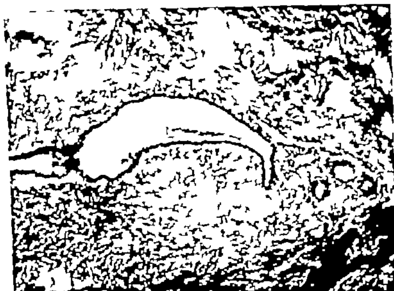


Fig. 7 From the same slide as Fig. 6. More deeply situated focus with more obvious endometrioid changes. Haem. + van Gieson. × 120 Slightly reduced.



Fig. 8 Area of well developed, flourishing endometriosis. Same slide as Fig. 6 and Fig. 7 Haem. + van Gieson × 120 Slightly reduced.



Fig. 9. Endometriotic cyst with papillary formations of the type occurring in the serous cystadenoma (right) Opposite wall shows endometriotic tissue (left) Haem + van Gieson. × 50 Slightly reduced.

metriosis consisting of epithelium and stromal tissue (Figs. 4 6 7 8)

Besides the morphologically indifferent epithelium which un doubtedly represents a fairly early state of metaplasia the mixed changes occasionally occurring in more advanced cases prove that different pathological processes in the ovary may be derived from common ætiological factors *i e* from metaplasia in the germinal epithelium. An illustrative example of such mixed forms was recently observed by the author Both ovaries of a woman aged 41 showed an unusual abundance of endometriosis the columnar epithelium of which was strongly proliferating, forming in many places solid islets with squamous metaplasia In a cyst formation elsewhere lined by simple columnar epithelium of endometrioid type papillary formations were found emanating from the inner surface of the cyst. The structure of these papillary excrescences corresponded completely with those occurring in the papillary serous cystadenoma (Fig 9)

The observations described above beyond doubt speak in favour of the genesis of ovarian endometriosis *in loco* through metaplasia in the germinal epithelium. This becomes evident not only through the gradually progressing changes in the inclusion cysts, but also through findings like those in the case quoted with papillary formations of serous type in *endometriotic* tissue. As such potencies of differentiation have never been demonstrated in the uterine endometrium they would scarcely be expected in transplants originating directly from it. In this connection it may be mentioned that Hughesdon (1949) published one similar case but, as regards the main type of differentiation, having the mixed changes in reverse. An adenofibroma of the ovary showed partial differentiation to endometriosis.

The study of the metaplastic changes in the epithelium of inclusion cysts seems to show that these changes essentially run along two lines. One of these, which perhaps could be named the tumour line, is characterized by columnar epithelium similar to that in the Fallopian tube and typical of the epithelium in the serous cystadenoma. Less frequently columnar epithelium of pseudomucinous type occurs. The stromal tissue adjacent to the epithelium generally shows no differentiation in a proper sense, only occasionally a non-specific condensation. The other line, as a suggestion called the endometriosis line, goes through more or less intermediate changes to structures typical of endometriosis in the epithelium as well as the stroma. That both potencies may sometimes simultaneously characterize a pathological process in the ovary is shown by the afore-mentioned mixed changes as well as by the fact that endometrioid and serous or pseudomucinous characters can sometimes be distinguished in different parts of an ovarian adenocarcinoma. The metaplasia in the stromal tissue is not necessarily interpreted as a secondary process, induced by the epithelial changes. In all probability the metaplasia in the epithelium runs parallel to that in the histogenetically closely related ovarian stroma (Gruenwald, 1943).

The possibility can not be excluded that the changes discussed in this paper may also originate from the germinal epithelium without the intermediary of inclusion cysts.

SUMMARY

Histological examination of metaplastic changes in inclusion cysts of the ovary shows in addition to indifferent columnar epithelium presumably representing an initial stage differentiation either *towards serous or pseudomucinous epithelium* ("tumour line") or towards endometrioid structures ("endometriosis line") The observations undoubtedly speak in favour of genesis of endometriosis *in loco* by means of metaplasia This opinion is supported by the occurrence of mixed changes, *e g* partial differentiation into papillary serous cystadenoma in otherwise typical endometriotic cysts Corresponding potencies of differentiation have never been demonstrated in the uterine endometrium proper

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PROTON MAGNETIC RESONANCE STUDIES ON THE STRUCTURE OF WATER IN THE MYOMETRIUM[1]

BY

ERIK ODEBLAD AND AXEL INGELMAN SUNDBERG

The physico-chemical state of water in the smooth muscle of the uterus at rest and during contraction is to a great extent still unknown. As information of the types of bonding and movement of water molecules in tissues can be gained from investigations with proton magnetic resonance (Odeblad, 1959 and references contained therein) this method was applied on uterine musculature from animals and humans. As in other organs part of the water is intracellular and part is extracellular (Fig. 1) It might be expected that only the intracellular fraction would be the subject of primary changes related to muscular contraction. Other complications arise from the fact that pieces of uterus also contain other tissues notably connective tissue which are not directly engaged in the process of contraction. No great effect of contraction on the magnetic resonance signals therefore can be expected

Material

A total of 76 mice and 19 guinea pigs were used in this study Uterine slices were taken from mice during different phases of the ovarian cycle during the last week of gestation and after delivery Castrated animals were also investigated 16 days after bilateral oophorectomy

[1] Supported by grants from the Swedish Medical Research Council and the Foundation Therese and Johan Anderssons Minne.

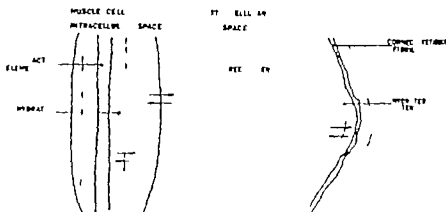


Fig. 1 A schematic diagram of the water phases in myometrium. There are phases with free water inside and outside the muscle cells. Hydrated water is indicated on the contractile elements and on connective tissue fibrils. Water exchange between free and hydrated phases is indicated by arrows.

As in intact mice the rapid variations associated with the sexual cycle may cause differences in the NMR findings a standard hormonal condition had to be selected for reference For this purpose castrated animals were used. Each received 1 μg of oestradiol monobenzoate every second day for five doses beginning 14 days after castration and the samples were taken on the 4th day after castration Some castrated guinea pigs received a single injection of 200 μg oestradiol monobenzoate on the 14th day and were investigated on the 23rd day A control series, injected with only sesam oil did not show any signs of oestrogenic effects. The number of animals in each group is seen from Tables I–IV

Normal myometrium obtained during the proliferative phase was taken from five patients operated upon for extrauterine diseases In two cases normal myometrium was also obtained at Caesarean section in patients with term pregnancies.

Methods

This investigation with NMR was undertaken at the proton resonance frequency of 16 7 Mc/sec in a field of 3 9 kilogauss. Some control experiments were also made at 28 9 Mc/sec and at 6 8 kilogauss in a new spectrometer completed during the pro-

Table I. Proton Line Widths in c/sec for Myometrial Samples

Treatment or Phase	Mice Average ± S.E.	No. of Animals	Guinea pigs Average	No. of Animals	Humans Average	No. of Patients
Castrated	29±3	8	29	7		
Castrated + E2	22±	1	9	6		
Œstric	22±				20	5
Anœstric	56±3	9	30	6		
Pregnant (near term)	±	9			7	
Puerperal (5—7 d)	29±6					

T ble II Signal Areas in Per Cent of Corresponding Water Signal

Treatment or Phase	Mice Average ± S.E.	No. of Animals	Guinea Pigs Average	No. of Animals
Castrated	65±4	3	62	5
Castrated + E2	80±4	5	79	6
Œstric	72±7			
Anœstric	66±8	8	6	5
Pregnant (near term)	8	5		
Puerperal (5—7 d)	65	6		

Tabl III Shifts in ppm from Water Reference

Treatment	Mice Average ± S.E.	No. of Animals	Guinea Pigs Average	No. of Animals
Castrated	− 04 ± 02	9	− 00	6
Castrated E2	− ± 02		− 08	6

T bl IV Relaxation Times in Seconds for Mic

Treatment	T Average S.E.	No. of Animals	T Average S.E.	No. of Animals	T/T
Castrated	6 ±	9	06 _ 0 02		
Castrated E	8 + 0	9	± 05		4

gress of this work, but of similar construction to the first spectrometer which was described in detail previously (Odeblad, 1961) The resolution of the spectrometers was 2 c/sec. The samples amounted to about 20 mg and were examined at +21 C. The measurements were made as soon as possible after excision from fresh uterine tissue The NMR signals were recorded re

peatedly about three times every minute for at least five minutes, in some cases for about 30 minutes in order to study the periodic changes in the signals occurring in association with eventual myometrial contractions. In some experiments drugs were added to the sample in amounts of 1 μg for adrenaline 2 μg for methylergobasin 20 μg for papaverine and 0 01 I U for oxytocin. The drug was dissolved in a negligible amount of saline introduced with the aid of a capillary tube Only one drug was added in each experiment.

The determination of the position of the NMR signal was performed with external calibration using a mixture of equal volumes of H O and CH_3COOH. The standard deviation of this determination amounted to $\pm$0 04 ppm (Odeblad, 1959) The determination of signal area was measured relative to the equivalent signal area of a pure water sample and was expressed as a percentage of water the standard deviation amounting to about 6 per cent The signal width defined as the width at half maximum amplitude and expressed in c/sec was measured by standard side-band audiomodulation techniques the standard error being $\pm$3 c/sec

Proton magnetic relaxation times were also measured T by observation of the signal growth after application of a strong (saturation) radiofrequency pulse at the resonance frequency and T by using the technique of Gabillard (1951) using the repetition of signals with different intervals and the measurement of regrowth of interference wiggles. For further details of technique consult the book by Andrew (1955)

Interpretation of NMR data

In order to be of biological interest, the nuclear magnetic resonance data must be translatable into a biologically intelligible language

The basic equation for the NMR frequency is

$$v = \gamma \quad H$$

where γ is the magnetogyric ratio for the particular nucleus and is a constant factor for each isotope with magnetic nuclei. The

field H is the magnetic field strength at the location of the nucleus and is given by

$$H = H_o (1 - \sigma)$$

where H_o is the bulk magnetic field supplied by the magnet and σ is the electronic shielding factor which depends on the chemical bonding and physico-chemical state of the resonating nucleus For different values of σ H_o has to be given different values in order to bring the particular isotope into the nuclear resonance condition. This gives rise to different shifts for protons within a range of a few ppm of the bulk magnetic field. In particular if water is bound by hydrogen bridges, its resonance is shifted to lower fields (H_o) If however the hydrogen bridges are broken up resonance occurs at higher fields. Such breaking of the normal bridge structure of water occurs with electric dipole binding of water

The protons in any sample occupy and are distributed between two allowed quantization states in a magnetic field. The difference between these populations is very small but sufficient, and necessary for the induction of a nuclear resonance signal. It is suppressed by application of a strong resonance radiation intensity After that the distribution returns to its original value with the time constant T (spin-lattice relaxation time) Another time constant T (spin-spin interaction time) describes the mutual exchange rate of magnetic energy between neighbouring nuclear spins. In true liquids T and T are of similar magnitude, for pure water about 2 sec. In more viscous solutions both T and T decrease If the locking of molecules in certain positions continues, and the viscous state is converted into a solid state, T remains short, about 10 sec, but T will rise again to values of the order of seconds. If the quotient T_1/T significantly exceeds unity this fact will indicate an admixture of solid state to the sample investigated. Both T and T will also decrease if paramagnetic impurities are present in the sample (e g certain enzymes)

In a liquid with a single NMR line the line width of the signal is equal to $1/\pi$ T_2. If the time width exceeds this value the actual broadening may be caused by the superposition of partial, unresolved lines provided no instrumental line-broadening effects come into play

peatedly about three times every minute for at least five minutes, in some cases for about 30 minutes in order to study the periodic changes in the signals occurring in association with eventual myometrial contractions In some experiments drugs were added to the sample in amounts of 1 μg for adrenaline 2 μg for methylergobasin 20 μg for papaverine and 0.01 I U for oxytocin. The drug was dissolved in a negligible amount of saline introduced with the aid of a capillary tube Only one drug was added in each experiment

The determination of the position of the NMR signal was performed with external calibration using a mixture of equal volumes of H_2O and CH_3COOH The standard deviation of this determination amounted to ±0 04 ppm (Odeblad, 1959) The determination of signal area was measured relative to the equivalent signal area of a pure water sample and was expressed as a percentage of water the standard deviation amounting to about 6 per cent The signal width, defined as the width at half maximum amplitude and expressed in c/sec was measured by standard side-band audiomodulation techniques the standard error being ±3 c/sec

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$$\nu = \gamma \; H$$

where ν is the magnetogyric ratio for the particular nucleus and is a constant factor for each isotope with magnetic nuclei The

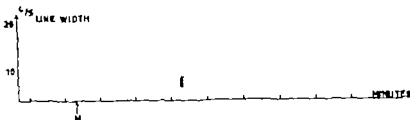


Fig. 3 The line width of a myometrial sample plotted against time during a run over more than minutes. Methylergobasin added at M. About two minutes later the NMR line begins to broaden from about 12 to about 7 c/sec, (open dots) The line is then split into two lines, persisting during three records. The separation between the doublet lines is shown by the filled dots, and their individual line widths by the crosses (x) Thereafter the two signals merge into single broad line which gradually approaches the original line width.

oxytocin were added, the same periodic changes were observed. No qualitative influence of adrenaline or papaverine was demonstrated

The results found in guinea pigs and humans are in agreement with those described for mice.

Discussion

It is generally believed that part of the tissue water is free and part of the water hydrated on the inorganic and organic matter in the tissues. There are several observations in the present study indicating that a considerable fraction of the tissue water in the myometrium is hydrated. Thus the significant negative shift in connection with the high relation T_1/T is an indication for hydration. The shift alone does not conclusively indicate hydration because paramagnetic substances may give rise to such a shift The fact, that the negative shift seems to increase but the quotient T_1/T to decrease following oestrogen treatment of castrates indicates in fact, that there is more than one factor involved in bringing about the observed effects (Tables III and IV)

The line widths generally exceed the value expected from T by a factor of at least 10 This may be caused by the existence

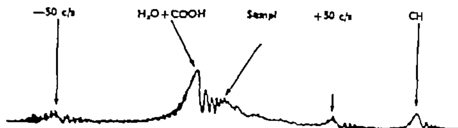


Fig 2. A typical record of NMR signals arising from the myometrial sample the calibration substance and the audiomodulation. The H_2O and OH-group protons exchange extremely rapidly and give rise to a single peak as indicated. The distance between this peak and the CH_3 peak is about 3 5 ppm. Magnetic field increasing to the right.

By conducting an analysis along these lines given above the NMR data *can be transferred into biologically useful information.*

Results

The results are listed in tables I–IV The proton resonance line under the standard hormonal conditions described above consists of a single line the area of which is about 80 per cent of the corresponding signal for pure water As the amount of dry substance in myometrium according to our measurements, amounts to about 22 per cent under these conditions all the tissue water is embraced within the observed resonance line The shift of the line is –0 10 ± 0 02 (S.E.) from pure water An example of a recording is shown in Fig 2 The line width is in average 22 c/sec the standard error being ± 5 c/sec. The spin-lattice relaxation time is 0 9 ± 0 2 sec and the spin spin interaction time 0 2 ± 0 05 sec the quotient T_1/T thus being greater than unity It should also be remarked that the line width expected *from T is only about 2 c/sec in disagreement* with the observed value of 22 c/sec. Therefore some additional line *broadening mechanism must exist.*

When single pieces of myometrium are subjected to recording during time intervals of the order of 30 minutes in some cases periodic changes occur in line width which are believed to reflect changes associated with the muscle contractions *per se* Line split also appears (Fig 3) When methylergobasin or

contractile activity in the sample and in some cases splitting of the NMR signals was observed also The interpretation of the results obtained indicates that a considerable degree of hydration of the tissue water must exist in myometrium and that this hydration could, to a considerable extent, be effected by hydrogen bonding. The hydration seems to be complex a large part, how ever being associated with the plain muscle cell itself

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of partially split fractions of water in myometrium, thus indicat ing the existence of a complex state of hydration

Regarding the mechanism of hydration no definite conclusions can be drawn from our experiments but the average negative shifting may agree with the assumption that hydrogen bonds constitute an important mechanism of water binding in myometrial tissue This result when compared with other tissues (Odeblad and Forsslund 1962) confirms the opinion, that the hydration mechanisms may be different in different organs.

The periodic changes in the appearance of the NMR lines (broadening and splitting) which were sometimes observed, probably are in some way or other related to the contractions of the muscle cells This observation indicates that rather a large part of the hydration really occurs on the contractile elements of the muscle or is otherwise connected to the muscle cells. Periodic changes were however observed only in about one out of six samples investigated for a sufficiently long time This fact is quite reasonable The appearance of observable changes in the NMR records implies that synchronous contractions occur in the largest part of the sample If non-synchronized motor activity occurs a mixture of narrow broadened and split NMR signals will occur the net effect being only a non-structured broad signal. In spite of the greatest care during preparation some samples may have been damaged impairing the contractibility of the muscle cells. *However the fact, that line broadening and splitting* has been observed indicates that changes may occur in hydration or permeability of myometrial cells during the muscular contraction cycle Attempts are under way to determine the time relation between contraction and NMR changes observed.

SUMMARY

Proton magnetic resonance line widths line positions, signal areas and relaxation times were measured at 16 7 and 28 9 Mc/sec in myometrial slices from mice guinea pigs and humans. The line widths were smallest under oestrogenic influence and larger under castrated or anoestric conditions Spontaneous variation occurred in line width which suggested a relationship to the

1) Nous ne nous sommes plus limités aux cas d'avortement habituel au sens restreint, mais avons pris sous le nom d'*avortement récidivant* tous les cas où il y avait eu 2 avortements consécutifs et non 3.

2°) Notre statistique est certainement faussée par le fait qu'*on nous envoie spécialement* des cas suspects de béance ou de malformation.

Cette nouvelle série porte sur 333 cas observés dans notre clientèle privée entre 1950 et 1963 et ayant eu une hystérographie. Or *nous avons soumis systématiquement à l'hystérographie toute femme consultant pour avortement récidivant* si elle n'était pas déjà enceinte lors de sa consultation.

Sur cette nouvelle série, nous trouvons :
- 64 malformations avec bifidité
- 39 hypoplasies corporéales
- 114 béances de l'isthme
- 18 synéchies traumatiques
- 13 autres anomalies

et seulement 85 utérus absolument normaux.

En fait, *à notre grande surprise*, les pourcentages dans les deux séries sont très voisins, et doivent donc correspondre à une réalité objective (Tableau 1).

Technique de l'Hystérographie

Quelques points dans la technique de l'hystérographie méritent d'être discutés.

) – *Sa date dans le cycle*

Lors de notre travail de 1950, nous préconisions dans ces cas, l'hystérographie en phase lutéale, parce qu'elle nous paraissait pouvoir donner des renseignements plus intéressants sur le *comportement musculaire de l'utérus*, puisque le comportement de la phase lutéale est très analogue à celui des premiers mois de la gestation.

Cela reste vrai pour une étude scientifique des cas sélectionnés.

Palmer Raoul, Pulsford, Jean, et Proust Josette Acta obst. et gynec. scandinav 44, 16, 1965.
Travail de la Clinique Gynécologique (Professor Jacques Hugier)
Paris France

L HYSTÉROGRAPHIE DANS L AVORTEMENT RÉCIDIVANT

PAR

RAOUL PALMER, JEAN PULSFORD ET MLLE JOSETTE PROUST

L *étude radiologique systématique* de 375 cas d avortement récidivant nous a convaincu qu *un facteur local utérin existe dans une majorité des cas* et que *seule l'hystérographie* permet de le dépister et de l analyser utilement

On nous objectera que notre statistique est faussée par le fait que beaucoup de gynécologues nous envoient leurs cas de bifidité ou de béance pour avis ou pour opération

Cette objection est valable pour les dix dernières années mais ne l est pas pour notre travail princeps de 1950 à la Société Royale Belge portant sur nos 42 premiers cas à une époque ou personne ne savait que nous nous intéressions à ce problème et où nous avions radiographié systématiquement tous les cas ayant eu au minimum 3 avortements consécutifs (*Avortement habituel* au sens restrictif du terme) Il nous paraît donc important de rapporter d abord les résultats globaux de cette *statistique non sélective*

Sur 42 cas d avortement habituel, nous trouvons

- 7 malformations avec bifidité
- 7 hypoplasies indiscutables
- 15 fois une béance évidente de l'isthme
- *11 fois d autres anomalies indiscutables* et

7 fois seulement un utérus absolument normal.

Nous apportons aujourd'hui une statistique beaucoup plus étendue mais peut-être moins convaincante pour deux raisons

1) Nous ne nous sommes plus limités aux cas d'avortement habituel au sens restreint, mais avons pris sous le nom d'*avortement récidivant* tous les cas où il y avait eu 2 avortements consécutifs et non 3.

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Cela est vrai pour une étude scientifique des cas sélectionnés.

Tableau 1 Tableau statistique des lésions utérines décelées par l'hystérographie A) dans 42 cas d'avortement habituel (série 1942-1950) B) dans 333 cas d avortement récidivant (série 1951-1963)

	AV HABIT 1942—1950	AV RÉCID 95 —1963	ENSEMBLE	%
Nombre de cas	42	333	385	
Malformations	7	64	72	18,9
Hypoplasies	7	39	46	12,2
Fibromyomes	5	7	12	3,2
Flexions etc	6	6	12	3,2
Béances isthm.	15	114	129	34,4
Synéchies	—	18	18	4,8
Normaux	7	85	92	24,8

Par contre dans la pratique cela se heurte à quelques difficultés 1°) il faut injecter beaucoup *plus de produit* de contraste pour remplir certainement l utérus 2°) il faut conseilller d *éviter une fécondation* ce mois-là 3) il est plus *difficile de comparer* les aspects et les dimensions à ceux des autres utérus ou l'H S G a été faite dans la semaine post menstruelle

Aussi en pratique en dehors des cas sélectionnés, faisons nous actuellement ces hystérographies *comme les autres* entre le 8° et le 12 jour du cycle.

2) – *Le délai après la dernière fausse-couche*

Il ne faut pas faire la radiographie trop tôt, car l'isthme et la cavité corporéale peuvent paraître plus larges qu'ils ne le seront 1 ou 2 mois plus tard

Il ne faut pas non plus trop attendre car on peut être pris de court par une nouvelle grossesse cela nous est arrivé 3 ou 4 fois

Aussi demandons nous d éviter une grossesse pendant 2 mois et faisons l'hystérographie le 3ème mois aussitôt après les règles

3°) – *Le choix du produit de contraste*

Il faut qu'il soit *suffisamment visqueux* (au moins 100 centi poises) pour distendre d abord l utérus avant de filer dans les trompes et le péritoine

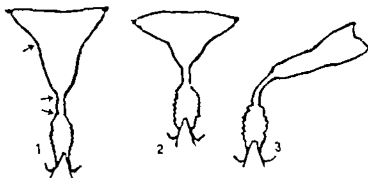


Fig. *Images d'un utérus normal chez une nullipare adulte*
(dimension réduite de moitié)

En 1 cliché de face avec *forte traction* sur le col, chacun des 3 bords de la cavité corporéale a environ 5 cm. de long; les encoches des bords latéraux permettent de bien délimiter le fond d'avec l'entonnoir sus-isthmique. Deux autres encoches délimitent les extrémités supérieure et inférieure du canal isthmique.

En 2 cliché de face sans traction le fond apparaît fusiforme, l'entonnoir sus-isthmique et l'isthme sont raccourcis.

En 3, cliché de quasi-profil, montrant bien le caractère courbe du canal isthmique qui est nettement plus long que sur les clichés de face.

4°) – *La nécessité d'un contrôle réel des pressions*

Nous avons pu vérifier des erreurs (notamment dans le diagnostic radiologique des béances) par insuffisance de pression, lors de la prise des clichés.

Pour obtenir de façon certaine un *moule utérin vrai* il faut réaliser pendant la prise des *clichés de réplétion* (face et profil) une pression de *100 mm* de mercure dans l utérus.

5°) – *Le choix du type de canule*

Il est préférable chaque fois que celà est possible d'utiliser une *canule à embout court* qui injecte le produit au centre de la cavité cervicale de manière à avoir une bonne image de la cavité cervicale et de l'isthme

Nous avons observé plusieurs cas de *malformation* où l'on a cru à un *utérus unicorne* parce qu on avait utilisé une canule à embout long qui n avait injecté qu une des cornes.

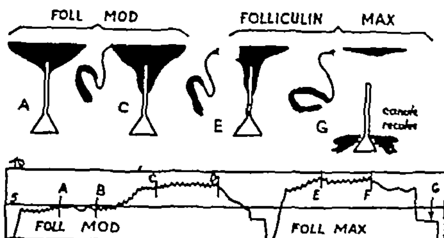


Fig 2 a. Radiomanométries en phases foll. mod. et foll. max.

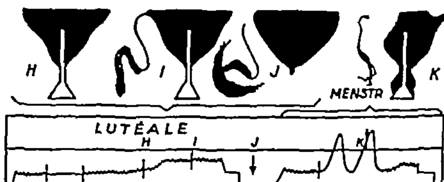


Fig 2 b Radiomanométries en phases lutéale et menstr

Fig 2. Variations physiologiques au cours du cycle menstruel.

La *ventouse suédoise* VUC nous a donné toute satisfaction

6°) – *La position pendant la prise des clichés* (Fig 1)

Nous prenons habituellement

– un *premier cliché* de face après l'injection de 1 ou 2 ml. pour ne pas risquer de méconnaître un polype ou un myome sous muqueux discret.

– un *2ème cliché* de face quand l'utérus est plein (pression à 100) et en tirant fortement sur le col pour étaler l'utérus dans un plan parallèle à celui du cliché

- un *cliché de profil ou mieux de quasi-profil* la femme faisant une rotation de 120° environ, de telle sorte que sur l'écran de télévision, l'utérus vienne s'inscrire dans la zone claire, large de 5 cm., entre les deux têtes fémorales. Ce cliché de profil est pris *sans tirer sur le col* puisqu'il doit indiquer la situation et l'orientation réelle de l'utérus dans la cavité pelvienne.
- un dernier cliché de face *cliché d'évacuation* pris aussitôt après l'ablation des appareils

L'interprétation

Reprenons maintenant quelques points de l'*interprétation* de ces hystérographies dans leur rapport avec l'avortement récidivant.

1) - *Rappel physiologique* (Fig. 2)

En phase folliculaire à la date habituelle de l'hystérosalpingographie l'utérus normo-tonique se laisse injecter sous une pression de 40 à 60 mm. Hg la cavité corporéale admet de 3 à 5 cc de liquide opaque et ses bords sont plutôt concaves. Le canal *isthmique* est sur les clichés large de 3 à 4 mm. le *fond utérin* est large de 45 à 60 mm. et sur le cliché de traction la cavité a sensiblement la forme d'un triangle équilatéral. Dès qu'on retire les appareils l'*utérus se vide vers le vagin*

En phase lutéale au contraire le canal isthmique est étroit et contracté (il faut une pression de 70 à 80 mm. Hg. pour le forcer) la cavité corporéale admet de 5 à 8 cc avant que le liquide ne reflue par les trompes et quand on retire les appareils l'utérus ne se vide pas immédiatement, et se vide plus volontiers vers les trompes et le péritoine que vers le vagin.

Nous avons eu 4 fois l'occasion de réaliser une hystérographie *au cours du 2ème mois de la gestation*. Les faits caractéristiques sont les suivants

a le canal isthmique est long et étroit et il faut une pression élevée pour le franchir (plus de 80 mm. Hg.)

b La cavité corporéale est atone à bords convexes et la pression à son intérieur est très basse (moins de 15 mm. Hg)

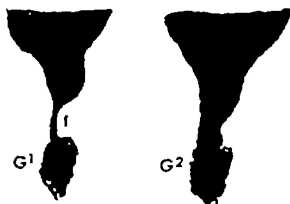


Fig 3 Béance de l'isthme (Cas Mintz) En G la béance est méconnue, mais la pression était insuffisante (50 mm. Hg.) En G la béance est évidente mais la pression était de 100 mm. Hg.

Il y a donc à la fois une atonie corporéale et une hypertonie isthmique ce sont là exagérées les modifications utérines de la phase lutéale du cycle

2) – *Béance de l'isthme*

Nous disons qu'il y a béance anatomique certaine de l isthme quand, sur le cliché de face le canal isthmique a une largeur de *1 cm ou plus*

Mais *la béance peut échapper à la radio* si on n a pas fait une pression suffisante En voici un cas démonstratif où l utérus paraît bien rempli pour une pression de 70 mm Hg avec un isthme dessiné d apparence normale *à 100 mm. Hg la béance est évidente*

Bien entendu, l'hystérographie *n est pas nécessaire* pour le diagnostic positif de béance anatomique de *l'isthme il suffit* pour le faire de pouvoir introduire sans résistance dans l utérus une bougie de Hégar n 8

L'hystérographie est néanmoins nécessaire pour vérifier la présence, assez fréquente d anomalies associées telles que

1) *Hypoplasie corporéale* (cas ULM, Fig 14)

2) *Synéchies traumatiques* après curettage (cas GLA, Fig 10) et MOR Fig 10)

3°) *bifidité associée* du fond (cas NEI Fig 13)

4) *diverticules traumatiques* au niveau de l'isthme visibles sur tout sur le cliché de profil (cas BOY Fig 11)

Rappelons le beau travail de Lepage et ses collaborateurs sur l'hystérographie après césarienne.

Ce n'est donc qu'après l'hystérographie et une étude attentive du col utérin et de l'anamnèse, qu'on pourra décider s'il faut envisager

- soit *une intervention avant grossesse* de trachelo-isthmorraphie élargie ou d'hystéroplastie avec isthmorraphie associée ou de traitements de synéchies intrautérines.
- soit, au contraire décider qu'on fera seulement un *cerclage* entre la 12° et la 15° semaine de la prochaine grossesse.

3) – *Malformations utérines*

Nous avons déjà signalé le risque de poser un *diagnostic erroné d'utérus unicorne* en utilisant un embout long.

Dans son rapport récent pour les Assises Françaises de Gynécologie Musset insiste sur la nécessité pour une classification correcte des malformations, de *confronter toujours les données de l'hystérographie d'une part et celles de la cœlioscopie ou de la laparotomie d'autre part*

La classification qu'il propose et qui nous paraît judicieuse distingue

a) – *les demi-matrices* (hémi-utérus) avec persistance plus ou moins complète de la dualité des canaux de Müller avec 3 sous-groupes (Fig 4)

- l'utérus bicorne bicervical (didelphe ou pseudo-didelphe (A)
- l'utérus bicorne unicervical (B)
- l'utérus unicorne (C)

Dans les utérus bicornes vrais, il est habituel que l'image radiologique des cornes diverge à angle assez obtus.

b) – *Les utérus cloisonnés*, avec absence de résorption plus ou moins complète de la cloison d'accollement des canaux de Müller où il distingue 4 groupes principaux

- *l'utérus cloisonné total* (D)
- *l'utérus cloisonné subtotal* Il y a au-dessus d'une cavité cervi-



Fig 4. Utérus malformés *par absence de fusion* des canaux de Müller. A. Utérus pseudo-didelphe B. Utérus bicorne vrai. C. Utérus unicorne vrai.

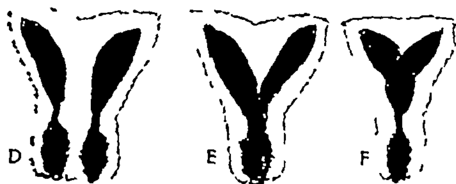


Fig 5 Utérus malformés par absence de résorption de la cloison médiane. D Utérus cloisonné complet. E. Utérus subseptus. F Utérus à fond bifide.

cale unique 2 demi-isthmes et 2 demi-corps, qui *divergent moins* que le bicorne unicervical vrai.

– *l utérus cloisonné corporéal* ou la cloison persiste sur presque toute la hauteur de l utérus (sub septus) (E)
ou sur une partie seulement (utérus à fond échancré ou à fond bifide (F)

D après notre expérience personnelle corroborée par celle de MM. Lacomme et Bret, *ce ne sont pas les degrés majeurs qui sont les plus nocifs*

Les utérus doubles les utérus bicornes vrais les utérus cloisonnés totaux ou subtotaux donnent plus souvent des accouchements prématurés que des avortements récidivants

Les variétés les plus dangereuses dans notre expérience sont l'*utérus subseptus* et l'*utérus à fond bifide* d'autant qu'il s'associe souvent à la bifidité du fond une béance congénitale de l'isthme.

Nous opérons ces cas par une variante personnelle de l'opération de Strassmann s'il y a eu 3 fausses-couches successives, ou 2 malgré un maximum de soins lors de la deuxième grossesse s'il y a béance associée de l'isthme, nous associons une isthmorraphie par avivement.

Si nous voyons la femme déjà enceinte au cours de sa deuxième grossesse et si le col paraît faible, nous pensons qu'on peut tenter le cerclage et associer un traitement de progestérone-retard à fortes doses nous avons ainsi obtenu un accouchement à 8 mois.

4°) – *Les hypoplasies utérines*

Nous venons d'écrire pour les Assises Françaises de Gynécologie un rapport sur les Hypoplasies utérines corporéales.

Nous avons étudié personnellement 2.200 dossiers de stérilité ou d'avortement comportant une hystérographie valable. Une hypoplasie indiscutable fut retrouvée dans 169 cas, soit 7,6 pour cent des femmes présentant un problème de stérilité.

Par contre *sur 301 cas d'avortements à répétition, l'hypoplasie était en cause dans 42 cas soit 14 pour cent*

Classiquement, le diagnostic d'hypoplasie utérine corporéale est basé outre *l'impression* d'utérus petit au toucher sur une *hystérométrie globale* donnant une longueur utérine inférieure à 7 1/4 ou sur une *hystérométrie différentielle* donnant une longueur corporéo-isthmique inférieure à 4 cm. Malheureusement, l'*orifice interne du col* n'est pas toujours perçu avec netteté et on ne peut compter de façon sûre sur l'hystérométrie globale que si on a une technique constante pour sa mesure (pince sur le col contact appuyé)

Seule l'hystérographie bien faite permet une étude valable des hypoplasies utérines, et de distinguer parmi elles 3 classes, dont le pronostic est nettement différent.

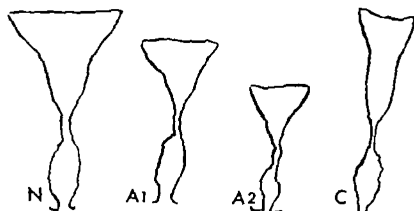


Fig 6. Utérus normal et utérus hypoplasiques.
La figure représente réduites de moitié, les images comparées de
N utérus normal.
A 1 hypoplasie corporéale simple
A 2 hypoplasie corporéale simple très accentuée
C hypoplasie corporéale allongée.

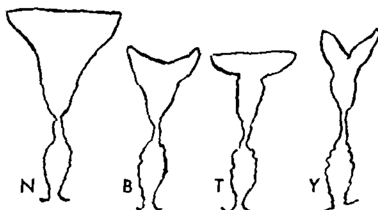


Fig 7 Utérus normal et utérus hypoplasiques malformés.
La figure représente également réduites de moitié les images comparées de:
N utérus normal.
B hypoplasie corporéale malformée à fond fortement concave.
T hypoplasie corporéale malformée, en forme de T majuscule.
Y hypoplasie corporéale malformée en forme d'Y majuscule

A) – *l'hypoplasie corporéale simple,* où la cavité corporéale a la forme normale d'un triangle équilatéral, mais de dimensions réduites souvent le col est par contre assez grand, mais ce n'est pas constant (Fig 6 A 1 et A 2)

B) – *L'hypoplasie corporéale malformée* groupant les utérus nettement hypoplasiques qui présentent un fond très arqué en forme d'un T majuscule ou d'un Y majuscule (Fig. 7 B T et Y)

C) – *L'hypoplasie corporéale allongée* où le fond est étroit, mais l'entonnoir sus-isthmique relativement long (Fig. 6 C)

Comme *critère radiologique mesurable* d'hypoplasie corporéale nous avons pris tous les cas où le fond utérin avait, sur le cliché de réplétion, *une largeur inférieure à 40 mm.* alors que le diamètre transversal de la clarté pelvienne avait de 155 à 175 mm.

Avec ces critères, sur 77 cas d'hypoplasie corporéale isolée (c'est à dire sans facteur tubaire ovarien ou masculin associée) 12 (15 %) restent stériles primaires.

- 9 (11 6 %) ont mené d'emblée à terme une grossesse.
- 7 (9 %) ont commencé par un accouchement prématuré.
- 42 (55 %) ont commencé par plusieurs fausses-couches spontanées de *1M1/2 à 4 M.*
- 17 de celles-ci (20 pour cent) ont jusqu'à présent, après 1 ou plusieurs fausses-couches mené une grossesse jusqu'au terme.

Au total, 32 femmes seulement (40 pour cent) ont eu un enfant vivant, dont 7 prématurés et parmi les 25 à terme 16 l'ont eu après un traitement intensif parfois prégravidique, plus souvent pergravidique

On peut conclure avec certitude que *l'hypoplasie utérine corporéale est une cause fréquente d'avortements spontanés à répétition* et qu'il y a une progression dans la *gravité du pronostic* depuis le type C. (H. C allongée) au type A. (H C simple) et surtout au type B (H C. malformée)

Cependant, dans aucun type le pronostic n'est obligatoirement désespéré puisque sur 26 cas du type malformé 6 finiront par *mener une grossesse jusqu'à viabilité* du produit.

Signalons aussi l'importance de l'hystérographie pour déceler *une béance associée de l'isthme* qui justifiera un cerclage si la grossesse a réussi à dépasser la 15ème semaine en bon état.



Fig. 8 Béance simple chez une grande multipare (5 ACC, puis 2 FC à 5 M 1/2)

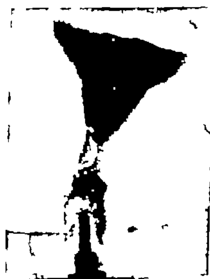


Fig. 9. Béance compliquée de synéchies au niveau de l'isthme.

5° – *Synéchies* (Adhérences intra-utérines)

Un groupe actuellement non négligeable, apparemment in connu ou méconnu en 1950 est constitué par les Avortements liés à des *adhérences intra-utérines après curettages*

Nous en avons récolté 18 cas dans les dix dernières années.

Nous considérons qu on peut poser le diagnostic ferme de synéchies par la radiographie si les images lacunaires sont à bords très nets et *invariables* avec le degré de réplétion Toutefois nous accepterions d ajouter à la classification de Toath un *degré zéro* pour les cas qui disparaissent soudain au moment de la réplétion maxima de l utérus et ne réapparaissent pas pendant l évacuation Il a pu s agir de synéchies muqueuses lâches qui auront cédé à la distension.

D après notre expérience les plus nocives sont les centrales ou voisines du centre à la base des cornes.

Par contre pour le traitement, les plus difficiles à attaquer sont celles qui font corps avec les bords, quelle que soit leur étendue

Ajoutons que nous les traitons habituellement par résection à la pince de Douay (pince à biopsie emporte-pièce) suivie de pose d un ballonnet qu on laisse en place une semaine sous antibiotiques et folliculine

Une radiographie de contrôle est pratiquée un mois plus tard et il y a parfois lieu à un complément de résection à la suite de celle-ci

6°) – *Autres anomalies utérines*

1 – *Les fibro myomes* souvent invoqués autrefois (5 sur 42 en 1950) ne sont plus signalés que rarement (7 sur 333 en 1963) En fait, les cas ou ils ont été considérés comme la cause principale sont rares puisqu il n a été pratiqué que 3 myomectomies dont 2 ont été suivies d'accouchements à terme

2 – *Les hyperflexions utérines* sont également invoquées de façon de plus en plus rare mais le fait qu elles ne soient guère signalées à l occasion de l'hystérographie ne doit pas surprendre car la pince mise sur le col et la traction sur celle-ci font disparaître l'image du triangle utérin renversé et, par ailleurs,



Fig. 10. Béance très importante compliquée de 3 synéchies centrales et d
bifidité du fond utérin.



Fig. 11 Béance très importante compliquée de diverticules traumatiques d
l'isthme (Cliché de quasi profil)

le cliché de profil sans traction n'a été réalisé de façon systématique que dans les deux dernières années.

Nous avons 3 cas où, après ligamentopexie les grossesses suivantes sont allées à terme sans histoire.

3° – L'Hypercontractilité utérine dont nous faisions grand cas autrefois, lors des hystérosalpingographies au lipiodol, où des contractions semblaient parfois segmenter ou boudiner les images des cornes a presque disparu de l'écran depuis l'emploi des organo-iodés hydrosolubles, sans que nous arrivions très bien à comprendre cette différence.

Les seuls qui restent hypertoniques et apparemment irritables sont certains utérus hypoplasiques et notamment ceux en forme de T majuscule.

4° – Quant aux Polypes muqueux nous en avons rencontré plusieurs fois et les avons enlevés par pêche à la pince ou par curettage sans être pour cela convaincus qu'ils avaient un rôle dans les avortements récidivants.

Au total, l'étude radiographique systématique de ces 375 cas d'avortements récidivants nous a convaincu qu'il y avait un *facteur anatomique utérin préexistant* dans une majorité des cas. Cela ne veut pas dire dans notre esprit, ce facteur soit toujours essentiel ou principal.

En effet, dans notre expérience il est fréquent, dans les cas d'avortement récidivant, de mettre en évidence plusieurs facteurs associés les uns anatomiques les autres fonctionnels (*endocriniens* en particulier) d'autres enfin neuro-vasculaires et *psychosomatiques*.

Les facteurs neuro-vasculaires et psychosomatiques ont souvent, en particulier *un rôle déclenchant de l'avortement* sur un terrain utérin ou endocrinien défavorable On connaît le rôle particulièrement néfaste de l'angoisse chez ces femmes obsédées par la crainte d'une nouvelle fausse-couche et de tout ce *qui* peut la laisser présager On sait aussi le rôle déclenchant possible du coït avec orgasme

Tout ceci pour rappeler que *le fait d'avoir découvert un facteur anatomique utérin* ne doit pas faire arrêter les recherches



Fig 12. Gros myome sous-muqueux et béance modérée de l'isthme



Fig 13 Utérus bifide avec béance associée de l'isthme

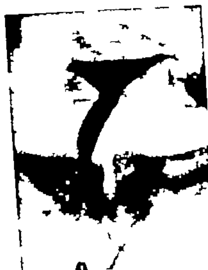


Fig. 4 Hypoplasie avec béance associée de l'isthme.



Fig. 5. Hypoplasie en T majuscule avec hyperantéflexion et légère béance de l'isthme (Cliché de 3 4)

des autres facteurs possibles dont les uns préexistent à la grossesse et dont d autres ne peuvent être décelés qu au moment de celle-ci

La connaissance plus *précise de la fréquence et des modalités* de la béance itshmique des malformations et des hypoplasies, *grâce à l hystérographie* représente un facteur certain de progrès dans le traitement de l avortement récidivant.

SUMMARY

The uterine factors in repeated abortion must be studied by *hysterography* (with manometric control pictures being taken at a pressure of 100 mm Hg one of them with maximal pull on the cervix, and one in profile position without any pull)

This systematic study of 375 cases has shown malformations (bifidity) in 18 9 per cent, uterine hypoplasia in 12.2 per cent, intra uterine adhesions (synechiae) in 4.8 per cent, sub-mucous fibroids in 3 2 per cent fixed retroflexion in 3.2 per cent and incompetence of isthmus in 34.4 per cent. The uterus was normal in 24.6 per cent only of this series

The authors believe that most cases of repeated abortion are due to a combination of several factors of which the uterine one is very frequent and often curable

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A NEW INSTRUMENT FOR HYSTEROSALPINGOGRAPHY

BY

LAURI RAURAMO

The most commonly used instruments for hysterosalpingography are still those based on the principle introduced by Schultze (1939). The principle common to all these instruments is closure of the cervix by a cone-shaped attachment. Interchangeable cone pieces whose size depends on the extent of laceration or dilatation must be used for cervices of different types. In Finland, Johansson (1956) developed a modification of Schultze's instrument with a flexible tip in lieu of a rigid one. In Schultze's and Johansson's equipment the instrument is attached to the cervix by means of toothed forceps. The cone is pressed tightly into the cervical canal using a screw thread to tighten the toothed forceps. When the toothed forceps have been in position for some time they may move dislodge to some degree and the closure of the cervical canal becomes incomplete. According to Johansson Schultze's original instrument carries the risk of perforation. Solla, Grönroos, Kauppila and Pyykönen (1962) on the other hand consider that the spiral tip of Johansson's instrument is weak and easily damaged. Similarly when the instruments age, the cock mechanism becomes worn and begins to leak, and the whole instrument usually has to be replaced.

Schultze and Erbslöh (1954) criticise Schultze's instrument as follows: "Ein Nachteil des von Schultze angegebenen Besteckes liegt in der Tatsache, dass das Instrument sehr schwer zu reinigen ist. Die drei auswechselbaren Uterusverschlusskegel

bestehen jeder aus 5 verschiedenen Teilen. Darunter befindet sich eine Gummidichtung, die bei längerer Benutzung durch das häufigere Sterilisieren leicht zerfällt. Wird der Uterusverschlusskegel nicht regelmässig auseinandergenommen und gereinigt, so findet man in der Tiefe reichlich alte und zersetzte Ölreste. Das 34 cm lange und höchstens 2 mm weite Füllrohr kann mechanisch überhaupt nicht gereinigt werden so dass sich auch hier leicht Rückstände des Kontrastöls ansammeln, die durch das Erhitzen des Instrumentes beim Auskochen in Sodalösung sich zersetzen
Screw-cannulas (Colvin 1939 Leech Wilkinson 1943 Fraser 1948) are easier to sterilise but fixation in the cervical canal by means of a screw thread is not always firm enough. A hysterosalpingography instrument which is held in position by a suction cup placed around the cervix has been evolved by Malmström. Fikentscher and Semm employ the same principle the suction cup and catheter are of polyethylene and are discarded after use

None of the hysterosalpingography instruments devised to date has proved ideal. The difficulties are especially noticeable when tonometry has to be performed at the same time as injection. The closure of the cervical canal may be incomplete when the instrument is fixed in the cervix by a separate forceps and when cone-shaped mouth pieces are used for the actual closure. During the injection and while the instrument is handled the forceps may give slightly at the point of fixation, resulting in seepage of the contrast medium into the vagina Definite closure of the cervical canal is essential for reliable results. Schultze and Erbslöh (1954) stated on this point "Für die praktische Diagnostik ist dagegen ein gewisser Überdruck unentbehrlich, der nur durch die Abdichtung des Uterus bei der Füllung zu erreichen ist. Nur eine sichere Abdichtung garantiert eine vollständige Auffüllung des Uterus nur diese bewahrt vor Fehldiagnosen. Die unvollkommene Auffüllung von Uterus und Tuben ist die Hauptquelle diagnostischer Fehler Die wichtigste Forderung, die an ein Füllgerät zur Hysterosalpingographie gestellt werden muss, ist daher die dass der Muttermund vollständig und sicher abgedichtet wird.

In 1960 at a meeting in Helsinki called Akademische Tagung the writer presented a film illustrating the possibilities of roentgen

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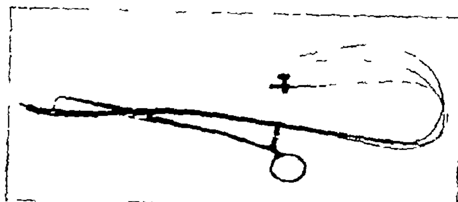
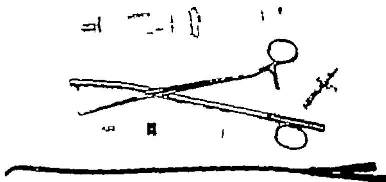


Fig 1

cinematography in gynaecologico-obstetrical examinations, especially in cases of urinary incontinence and hysterosalpingography He found that there was no single instrument perfectly suitable for hysterosalpingography An ordinary Foley catheter sutured with catgut to the cervix was used initially for these examinations. Fixing it in place, however was difficult because of the suturing involved. The writer therefore devised a hysterosalpingography instrument of metal with three channels. The instrument is seen in Fig 1 It consists of a toothed forceps one handle is tubular permitting the passage of 3 thin metal tubes. Through one of the tubes liquid is injected via an aperture at the tip of the instrument into the rubber ball. This closes the cervical canal effectively The apertures of the other 2 tubes open into the tip of the instrument The aperture of one of them is directed towards the centre of the cervical canal this tube is intended for tonometry and the aperture is so located that it will not be blocked by the mucosa of the cervical canal The third tube is for the injection of contrast medium into the uterus. The instrument is easily fixed in the cervix by a single movement of the hand and when the rubber ball has been fully inflated with liquid the preparations for salpingography are complete The tonometry tube is connected by a polyethylene tube of suitable thickness with the electronic manometer (Sanborn or Elema type) The tube for the injection of contrast medium and the tube leading to the rubber ball are connected with the syringes by a lockable intermediate piece

The instrument described above proved very suitable for



Fig

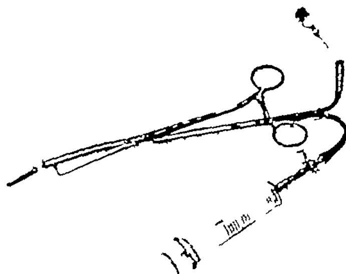


Fig 3

scientific investigations. Drawbacks that must be mentioned are the weakness of the joint between the rubber ball and metal tube and the relative difficulty in cleaning the long metal tubes. Insertion is very easy The instrument has been developed further for routine use as illustrated in Figs 2 and 3 The metal tubes with their small lumina have been replaced by an ordinary Foley catheter No 14 gauge in the other tubular half of the instrument. The catheter length must fit the tube closely and keep exactly in position between the inflatable rubber ball and the thickening at the upper end of the catheter The contrast medium syringe is connected with one canal of the catheter by a lockable intermediate piece with a cock and the rubber ball is filled from an injection needle and syringe via the rubber stopper at the end of the other catheter canal The amount of fluid to be injected into the rubber ball varies according to the size of the cervical canal The volume of the rubber ball of this type of catheter is 5 ml but an injection of about 3 ml usually suffices Care must be taken not to use too high a pressure Advantages of the instrument are ease of cleaning, simplicity low price and the fact that the metal parts can be sterilised separately from the rubber parts. No auxiliary forceps are needed for fixation The fixation is so secure that the patient can walk from the gynæcological examination room to the rœntgen room after the instrument has been placed in position It is easier to place the instrument in position on a gynæcological examination table equipped with the pertinent auxiliary apparatus It is not necessary in that case to fix leg supports on to the rœntgenological table The catheter is cleaned in the usual way unless it is disposable The instrument has so far been made in two thicknesses tube diameters 0 8 and 0 6 cm. For the latter diameter Foley's catheter No 10 gauge is used. A short description of the instrument in finnish has been presented by Rauramo (1963)

Figs 4 and 5 show some rœntgenograms taken with this instrument.

Finally a few practical points which should be remembered when using the instrument. The catheter lengths do not seem to be completely the same even when they are from the same manufacturer If the catheter is longer than the metal tube of the



Fig 4



Fig 5

instrument a piece of plastic tubing of the same diameter as the metal tube can be inserted around the catheter before inserting it into the tube When the rubber ball is fully inflated it tends to turn back in the lacerated cervix and to bulge out of the cervical canal if the catheter is just a little too long This is most easily prevented by closing the lacerated part of the cervical canal with an ordinary toothed forceps so that the cervical canal forms as it were a tube around the instrument. It is often more practicable to attach the instrument to the posterior lip of the cervix.

SUMMARY

A new hysterosalpingography instrument is described. The working principle of the instrument is the fixing of a metal tube in the cervical canal by a toothed forceps. A Foley catheter is passed via this metal tube The instrument has the following advantages

The catheter is disponsable

The instrument is easy to clean

The closure of the cervical canal is complete and safe

The stop cock forms a separate piece and is easy and cheap to renew when worn

The instrument is simple to position and remains securely in the cervix

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NEEDLE BIOPSY FROM UTERUS AND MYOMA DURING PERITONEOSCOPY

BY

STIG SAMUELSSON

A conchotome is generally used for biopsy of the female pelvic organs during peritoneoscopy However it is only suitable for superficially situated lesions such as peritoneal nodules tuberculosis and cancer endometriosis and similar For deep biopsy of the uterus and myomata alternative techniques are necessary

A biopsy needle has been used for deep biopsy from the liver for a considerable time (Iversen and Roholm, 1939 Kalk et al 1943, 1951 Silverman 1938 1954 Radner 1957 Stiefel 1961) Because of the construction of the needle either a cylindrical or a cone shaped tissue specimen is excised. This is detached from the base by torsion of the needle with the aid either of vacuum—the so-called aspiration technique—or by using the friction between the biopsy specimen and the wall of the needle (Silverman 1938 1954 Radner 1957)

Aspiration technique (for details see Samuelsson and Sjöstedt 1961) is used for biopsy from an organ rich in glands but with relatively little connective tissue With increased firmness of the tissue the method is less feasible Thus, with cirrhosis of the liver sometimes it can be difficult to obtain sufficient tissue for histopathological analysis. Experiments have proved the method to be impracticable for biopsy from the wall of the uterus and from myomata. Neither vacuum nor torsion is sufficient to detach any tissue

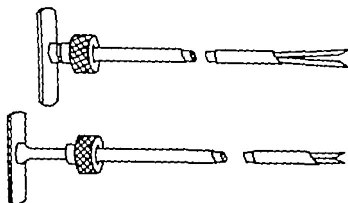


Fig 1 The Vim-Silverman needle

The Vim Silverman needle and the Radner needle excise cone-shaped pieces of tissue Because the base of the cone is compressed in the needle increased friction facilitates the detachment of the specimen (*Fig* 1)

Material and method

Fifteen patients were investigated. Indications for peritoneoscopy were as follows in five patients differential diagnosis between myoma and ovarian tumour to assess the need for surgical treatment in ten patients suspicion of adenomyosis where other methods of investigation (curettage and hysterography) gave negative results

Peritoneoscopy was carried out in the usual way (Sjövall 1962) The uterus was held firmly during the biopsy by pulling with forceps secured to the cervix. The biopsy instruments consist of a cannula with a trocar and Vim-Silverman needle The cannula with the trocar is inserted percutaneously through the abdominal wall in the pararectal line between umbilicus and symphysis and directed towards the fundus uteri or the myoma (*Fig* 2) The trocar is withdrawn and replaced by the Vim-Silverman needle which is introduced into the tissue for examination The cannula is slid down over the needle past its point. Needle and cannula are rotated and withdrawn One to three biopsies 3–4 cm in length were taken in each instance (*Fig.* 3)



Fig. The Vim-Silverman needle introduced into the uterus



Fig 3 Tissue specimen. The conical shape can be seen.

Results

Histological examination showed adenomyosis in one patient, benign fibromyoma in four and normal uterine wall in ten (*Fig* 4) Hysterectomy was performed later for various reasons on eight patients. In this way the diagnosis obtained by means of needle biopsy was confirmed in six instances. In two cases needle biopsy showed the uterine wall to be normal, but histological examination of the operation specimen revealed isolated centres of adenomyosis.

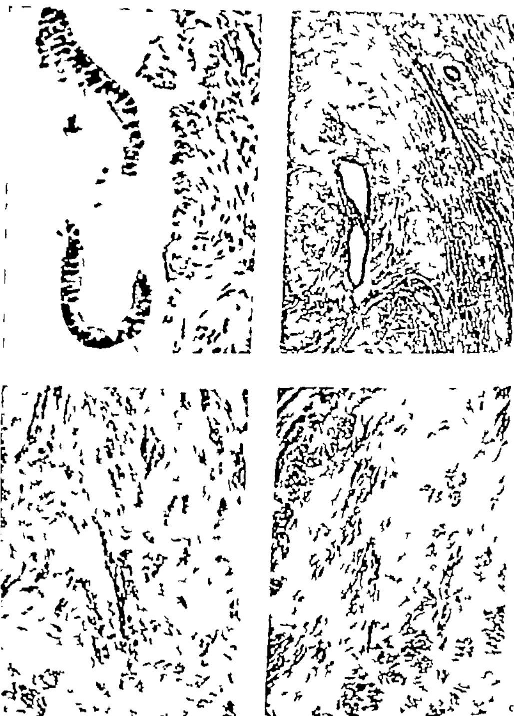


Fig. 4. a. Adenomyosis. Specimen obtained by the Vim-Silverman needle × 400 b. The operation specimen, × 63 c. Fibromyoma. Specimen obtained by the Vim-Silverman needle, × 160 d. The operation specimen, × 60.

In one of the patients needle biopsy was used to determine the nature of a tumour in the pelvis a women aged 73 with acute cholecystitis had bilateral pelvic tumours outside the uterus causing difficulty with micturition. If malignant ovarian tumours could be excluded it was intended to perform a cholecystectomy as a primary procedure At peritoneoscopy one mass was found to be subserous myoma but it was impossible to determine wheth er the other was also a myoma or an ovarian tumour Needle biopsy showed benign fibromyoma. The gallbladder operation was given priority and six months later the uterus and the two subserous myomas which were benign, were removed.

No complications occurred. The bleeding from the wound made by the biopsy was slight and ceased spontaneously after a few minutes.

Discussion

The Vim Silverman needle appears to be suitable for biopsy from the uterus and myomata at the time of peritoneoscopy The principle indication is clinical suspicion of adenomyosis or myomata

Clinical diagnosis of the presence of adenomyosis is often dif ficult. The objective methods for diagnosis are curettage and hysterography In a series comprising 82 patients with proven adenomyous endometrial deposits were found in the myomet rium at preoperative curettage in five patients and by contrast filled gland ducts at hysterography in 2/3 of the patients (Ploman, 1961 Norman, 1965) Needle biopsy from the wall of the uterus can supplement these methods of investigation.

Peritoneoscopy is a method frequently used for differential diagnosis between myomata and ovarian tumours where opera ti e treatment of myomata would not be required (Sjövall 1950 1962 Stamer 1953 Noyes 1954 Samuelsson and Sjövall 1964) In such cases, needle biopsy from the suspected myoma can supplement the other methods of investigation. Although needle biopsy only reveals the appearances in a small part of the myoma it gives a reasonable amount of information which is better than having no information at all.

SUMMARY

The Vim Silverman needle is suitable for deep biopsy from the uterus and myomata during peritoneoscopy A biopsy of this nature was performed on fifteen patients with myomata or suspected adenomyosis In six of eight patients operated on subsequently specimens obtained by needle biopsy showed histological changes representative of the changes subsequently found in the whole organ

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From the Department of Obstetrics and Gynaecology (Professor E. Schjött Rivers) University Clinic Oslo Norway

ADVANCED EXTRAUTERINE PREGNANCY

Report of Four Cases

BY

ANTON SUNDE

In general, there is hardly any difference of opinion regarding the treatment of extrauterine pregnancy Laparotomy as soon as the diagnosis is made is the method of choice, and this applies almost without exception in the early months of pregnancy because of the danger of intraperitoneal bleeding.

The question which has interested me, is whether this course is also indicated in the rare case of advanced extrauterine preg nancy These patients have passed the initial stages of acute danger The clinical picture they present is characterised by the fact that conditions in the peritoneal cavity have become stabilised. They are often symptom-free. Is it not justifiable provided the patient is under supervision to treat expectantly with the intention and hope that she may have an infant capable of survival?

I have treated four such cases in the past, and it was from my experience with the first three that I dared advise my last patient to continue the pregnancy despite the fact that by the middle trimester I was certain of the diagnosis of extrauterine pregnancy

This patient was 28 years old and unmarried She had had one previous child which had died, and one miscarriage. A cystic left ovary was removed three years previously She had become pregnant in October 1948 and was well until at two months gestation she had several attacks of pain associated with a feeling of faintness, and there was a little dark, almost black vaginal blood

SUMMARY

The Vim Silverman needle is suitable for deep biopsy from the uterus and myomata during peritoneoscopy A biopsy of this nature was performed on fifteen patients with myomata or suspected adenomyosis In six of eight patients operated on subsequently specimens obtained by needle biopsy showed histological changes representative of the changes subsequently found in the whole organ

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I have treated four such cases in the past, and it was from my experience with the first three that I dared advise my last patient to continue the pregnancy despite the fact that by the middle trimester I was certain of the diagnosis of extrauterine pregnancy

This patient was 28 years old and unmarried. She had had one previous child which had died, and one miscarriage. A cystic left ovary was removed three years previously She had become pregnant in October 1948 and was well until at two months gestation she had several attacks of pain associated with a feeling of faintness and there was a little dark, almost black vaginal blood

loss She was admitted to a surgical department where the diagnosis was considered to be a pregnancy with salpingitis, and Penicillin treatment was given

She came to the Gynæcological Department three weeks later when 23 weeks pregnant for further advice She felt well and was symptom free and the diagnosis was not difficult. The *posterior fornix was bulbing downwards and here it was possible* to feel the fœtal head with sutures and fontanelles The head was ballotable and there were obvious fœtal movements every time the palpating finger touched the head, indicating that the fœtus was lying freely mobile within its membranes The uterus could be palpated anteriorly a little enlarged and drawn high out of the pelvic cavity I explained the situation to the patient and *said that she could demand an immediate operation though this* probably would result in her being sterilised since she had only one ovary which now was almost certainly damaged, but I added also that it seemed to me justifiable to await events in the hope of giving her a live baby She wanted this very much and replied "Maybe then I will get married

Three months later she was again admitted two weeks before *term because of a few small labour pains she was otherwise* well. At laparotomy a live normal female infant was delivered, weighing 2580 g, and 44 cm long The fœtus had been lying fully protected within its intact membranes with the head in the Pouch of Douglas The placenta was easy to remove Its main blood supply was *from the ovarian vessels* in the right Infundibulo-pelvic ligament. The ovary could not be seen since the large placenta *appeared to originate from the right adnexa which* had to be removed to ensure hæmostasis. Postoperative progress was uneventful. The mother and child were examined six months later both were well and the child had developed normally

When I had advised this patient to continue her pregnancy it was because of my experience with three similar cases. The first case was many years ago and resulted in a living male infant weighing 2600 g and 46 cm long at the 36th week It had made quite satisfactory progress when seen one year later Here also it was easy to control the bleeding at the time of removal of the placenta. She was operated on soon after her admission to hos

pital, at which time she had reached the 36th week of her preg nancy

The next case was complicated by a twin pregnancy One inside the uterus the other outside. In this instance of both intra- and extra-uterine pregnancy the patient started labour pains and a laparotomy was performed at about the 30th week. The twins were alive at birth, but died shortly afterwards of prematurity weighing 1700 g and 1800 g respectively Again, it was not difficult to control the bleeding, although the situation was complicated because the extrauterine placenta was attached to the whole of the right half of the pelvis over the main iliac vessels. The patient returned the following year and had a normal delivery but being unmarried, she was very dissatisfied with both the hospital and her previous operation as sterilization was not performed which she had assumed

In the last case a term slightly macerated foetus weighing 3650 g was removed at laparotomy Here also it was easy to ensure hemostasis satisfactorily The major blood supply came from the right infundibulo-pelvic ligament and these vessels were ligated. The placenta was large weighing 1400 g. Postoperative progress was uneventful.

The common feature in all these four cases is of clinical interest. After the acute phase, with the typical symptoms of extrauterine pregnancy has passed these patients proceed into a more quies cent stage which may even be symptom-free. The operation findings show that this is due to the secondary development of the pregnancy within the abdomen resulting in reestablishment of the foetal blood supply

I therefore draw the conclusion that in these instances one is entitled to allow the pregnancy to continue especially if the patient wishes it, and provided that she is informed about the attendant risks involved.

The frightening descriptions in the literature about the diffi culties in controlling the bleeding at the time of operation in advanced extrauterine pregnancy seems to me to be a little exaggerated, particularly with the present availability of blood for massive transfusion.

Risks of malformation of the infants must not be exaggerated

either only one of these five infants had a little flattening of the head where it had been compressed in the Pouch of Douglas, and this was in the fortunately rare case of both intra and extra uterine pregnancy All the other four were normal in every way and two of them are known to have grown up satisfactorily

SUMMARY

On the basis of these four cases the author wishes to stress primarily the importance of removing the placenta at the time of laparotomy even though the anatomical conditions seem complicated, and despite the many warnings against this manoeuvre

The literature gives many examples of all the serious complications which arise when the placenta is left *in situ*

Secondly consideration is given to the justification in delaying the laparotomy but keeping the patient under strict supervision, in the hope of obtaining a *live infant, since it is usually* about the 20th week that peritoneal conditions become stabilised in a secondary abdominal pregnancy Two of these mothers had term infants of normal development.

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From the David Elder Infirmary Glasgow Scotland

THE PLACE OF SURGERY IN THE TREATMENT OF GENITAL TUBERCULOSIS IN WOMEN

BY

ARTHUR M. SUTHERLAND, M.D., F.R.F.P.S.G., F.R.C.O.G.

Introduction

In recent years there has been a widespread awakening of interest in the whole subject of genital tuberculosis in women. It is now generally realised that this condition is much more frequent than was previously thought and it is likely that this increase in the number of cases diagnosed is due in great part to the routine examination of the endometrium in patients with such complaints as infertility irregular uterine bleeding or amenorrhoea. Since the introduction of several potent anti tuberculous drugs, a considerable volume of literature has accumulated on the treatment of genital tuberculosis in women and very varied opinions have been expressed regarding the best methods of dealing with this condition.

Before the introduction of the anti-tuberculous drugs many forms of treatment had been employed with varying and often indifferent results. The choice then lay between surgery and various forms of conservative therapy Surgery was associated with considerable technical difficulty in many cases, a high incidence of post-operative complications, particularly of fistulae, and a substantial mortality The extent of the surgery carried out varied considerably with different workers and some merely employed simple laparotomy

Among the many forms of conservative treatment advocated were radiotherapy of different types, long-term sanatorium treatment with rest in bed, natural sunlight, ultra-violet radiation,

either only one of these five infants had a little flattening of the head where it had been compressed in the Pouch of Douglas, and this was in the fortunately rare case of both intra and extra uterine pregnancy All the other four were normal in every way and two of them are known to have grown up satisfactorily

SUMMARY

On the basis of these four cases the author wishes to stress *primarily the importance of removing the placenta at the time of* laparotomy even though the anatomical conditions seem complicated and despite the many warnings against this manœuvre.

The literature gives many examples of all the serious complications which arise when the placenta is left *in situ*.

Secondly, consideration is given to the justification in delaying the laparotomy but keeping the patient under strict supervision *in the hope of obtaining a live infant, since it is usually about* the 20th week that peritoneal conditions become stabilised in a secondary abdominal pregnancy Two of these mothers had term infants of normal development.

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or district or having ceased to attend in spite of repeated requests to do so

Of the remaining 225 cases, 44 (20 per cent) have so far been treated surgically in the writer's Unit. Histological proof of the tuberculous nature of the lesion was obtained in every case and bacteriological confirmation was established in 32. The average age of these patients was 26 years the youngest being aged 18 years and the oldest 38 Thirty three patients gave a history of a previous extra-genital tuberculous lesion X-ray of the chest revealed healed tuberculosis in 32 instances and active tuberculosis in one X ray of the abdomen showed calcified glands in 5 cases and bacteriological examination of the urine revealed tubercle bacilli in 2 instances In only 4 patients was no history or other evidence of extra-genital tuberculous infection found. All these operations were performed in the past 7 years.

Indications for operation Surgery was only used when drug treatment was judged to have failed and every patient treated surgically had had at least one drug course In the earlier cases in the series, short drug courses were employed, but with increasing experience the courses were lengthened. Since 1st January 1960 the minimum drug course administered to any patient has been 8 months of continuous treatment. The indications for surgery in the present series were as follows

( ) Abdominal pain persisting or recurring after drug treatment – 27 cases. In all of these patients the pain was associated with tenderness and thickening in one or both adnexal regions.

(2) The persistence or development of substantial adnexal masses following drug treatment—8 cases. In this group there was no complaint of abdominal pain. The possibility that the swellings might not be tuberculous in origin was always kept in mind. This danger is stressed by Wolskel and Barnett (1952) and by Snaith (1958) who reported cases of pelvic tuberculosis with masses which subsequently turned out to be due to carcinoma.

(3) Recurrence of endometrial infection following drug treatment—8 cases In this group the patients did not complain of abdominal pain and pelvic examination failed to reveal substantial adnexal masses.

(4) Excessive uterine bleeding in spite of drug treatment—1

carbon arc light, thermal treatment, pelvic diathermy tuberculin, various diets vitamins especially vitamin D calcium and injection of oxygen into the peritoneal cavity These various forms of treatment are considered in detail and the literature is fully reviewed in publications by Jameson (1935) Jedberg (1950) Sutherland (1950) Schaefer (1953) and Ylinen (1961)

After the introduction of the anti-tuberculous drugs many papers were published which dealt with the results of this form of treatment. For a time surgery was to a great extent in abeyance and attention was focused almost entirely on the new drugs. At first, the courses employed were short but later in the light of experience especially in the treatment of extragenital tuberculosis much longer courses were generally used. Extensive papers dealing with this aspect of the subject and reviewing the relevant literature were published by a number of writers including Jedberg (1950) Liljedahl and Rydén (1951) Rydén (1953 1958) Schaefer (1955, 1956 1959) Bret and Legros (1956) Sutherland (1958 1960) and Ylinen (1961)

As time went on it became apparent that drug treatment, especially in short term courses, was by no means always effective and more attention was again paid to the place of surgery This question has however received relatively little attention in the literature in recent years Snaith (1958) considered this matter in some detail and came to the conclusion that antibiotic treatment is the first line of choice in all cases of pelvic tuberculosis but that there is still a place for surgery especially after antibiotic therapy has failed to produce complete resolution of symptoms.

Present Series

An analysis has been made of the patients treated surgically out of a total of 446 cases of proved genital tuberculosis in women investigated and treated under the direction of the writer between 1st December 1950 and 30th June 1964 Ninety two of these patients were in other hospitals in Glasgow and were not under the control of the writer as far as surgery is concerned. One patient died of bronchial carcinoma In 128 further cases the follow-up is incomplete these patients having left the country

with tubo-ovarian masses at the time of operation, he advocated removal of the uterus tubes and ovaries, irrespective of the age of the patient. The case that he put forward for radical surgery is most convincing and the writer is in full agreement with his views. The cases in the present series treated surgically would all be classed as advanced according to his criteria.

Snaith (1958) said that radical surgery is desirable in any proved case of pelvic tuberculosis in which laparotomy becomes necessary but he leaves some ovarian tissue if it is apparently healthy Other writers favouring radical measures in such cases include Greenhill (1935, 1958–59) Stallworthy (1952, 1963) and Borrow and Batts (1952) On the other hand, restricted surgery is perferred by many including Liljedahl and Rydén (1951) Rydén (1958) Jedberg (1950 1956) Bret and Legros (1956) and Ylinen (1961)

Operative technique The most striking feature of the operations in the present series was the fact that, in general, they were less difficult than had been anticipated. While frequently time-consuming, these operations were usually relatively easy to perform, in marked contrast to the extreme difficulty encountered in the past. It is almost certain that this is due to the fact that all patients had previously received one or more courses of anti-tuberculous drugs in addition to the pre-operative drug programme In no case was it found necessary to leave any part of the uterus or adnexa because of technical difficulty

While adhesions were invariably present and usually widespread, they could be separated fairly easily either by sharp dissection or more often by blunt dissection with the gloved finger Provided that any pressure used is exerted towards the uterus or broad ligaments and away from bowel or adnexa no damage is likely to result. Where location of the pelvic structures is difficult, an initial move which has proved valuable is to locate and free the fundus of the uterus and insert a traction suture at each angle An interesting point about these cases was the fact that the bladder was almost invariably very high in position, sometimes coming to within less than an inch of the fundus of the uterus.

Post-operative complications. Three patients developed wound infection one of these being slight. Two patients required resuture

case Curettage was carried out on several occasions on this patient and each specimen showed well-marked cystic glandular hyperplasia In the writer's experience, all other patients with abnormal uterine bleeding and genital tuberculosis have shown a return to a normal menstrual cycle following drug treatment. *Pre-operative treatment* Before resorting to surgery everything possible was done to exclude active extra genital tuberculosis including X-ray of chest, examination by a chest physician and bacteriological examination of the urine for tubercle bacilli. If such a lesion was found it was treated before considering surgery for the pelvic condition All patients were admitted to hospital at least one to two weeks before operation and the operation was timed for the middle of the menstrual cycle. Occasionally where pain was severe, a spell of several or even many weeks in hospital was found necessary

On admission to hospital, each patient was started on a pre-operative course of anti tuberculous drugs Streptomycin PAS and isoniazid were all employed as a rule, unless there had been previous sensitivity to any of these in which case the offending drug was omitted. This drug course was continued after operation the precise drug combination and dosage depending on the individual case but the course being given for a total of at least six months and for at least one year if activity was found in the tissue removed at operation.

Operation performed. Total hysterectomy with removal of both tubes and ovaries was performed by the abdominal route in every case irrespective of the age of the patient. Consideration was always given to the question of conserving ovarian tissue but the ovaries in the patients treated surgically to date were invariably unhealthy and adherent to surrounding structures and it was considered that conservation of such ovarian tissue would not be in the best interests of the patients.

Considerable differences of opinion exist to-day on the question of the extent of surgery in patients with pelvic tuberculosis. Some writers favour removal of the uterus tubes and ovaries while others prefer restricted surgery conserving the ovaries and sometimes also the uterus Schaefer (1955) discussed this question in some detail. In what he termed advanced pelvic tuberculosis

SUMMARY

An analysis has been made of 44 patients with genital tuberculosis who were treated by surgery after failure of drug treatment. The indications for operation were pain (27 cases) pelvic masses (8 cases) endometrial recurrence (8 cases) and uterine bleeding (1 case) All operations were performed under drug cover Total hysterectomy with removal of both tubes and ovaries was carried out in every case. No patient developed a fistula and there were no deaths. The results at follow up were satisfactory in terms of relief of symptoms and return to normal life

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of the vaginal vault from below because of bleeding and one developed porphyria. There was one case of deep venous thrombosis, one of pneumonia and one of intestinal obstruction 6 months after operation due to an upper abdominal adhesion. There were no deaths.

Pathology. All specimens removed at operation were examined histologically and evidence of active tuberculosis was found in 19 (43 per cent). The details are as follows —

One or both tubes	10 cases
Both tubes and endometrium	3 cases
One ovary	2 cases
Endometrium	2 cases
One tube and ovary	1 case
Both tubes and ovaries, endometrium, myometrium	1 case

The discovery of ovarian involvement in 4 cases is interesting, particularly in view of the fact that the ovaries in these patients appeared to be no more unhealthy at operation than those in most of the others. This finding supports the view that removal of the ovaries is justified in advanced pelvic tuberculosis treated surgically. An attempt was made in all but one case to obtain bacteriological confirmation but this was successful in only one instance. Of the remaining 18 with positive histology bacteriological proof was obtained before the start of treatment in 15.

Results. The results of surgical treatment were generally satisfactory. One patient left the country shortly after leaving hospital. The average duration of follow up in the others was 2 years and 8 months. All of these patients remained well and were able to lead a normal life in every way. In those cases in which the operation was performed primarily because of pain, in every instance this was completely relieved. It is thus apparent that operation is equally successful in curing pain irrespective of whether the condition is subsequently found to be histologically active or not. Menopausal symptoms varied considerably in individual patients but where these were severe, a 15 mgm. oestradiol implant was found to be very effective. In every case the abdominal wound was found to be well healed at follow-up and on vaginal examination the vaginal vault was found to be healthy.

Material

By special questionnaires data were collected concerning 27 522 deliveries in Department II of Obstetrics and Gynaecology of Helsinki University Central Hospital from the years 1950–1960 (Series 1) Furthermore data were collected concerning all deliv eries in maternity hospitals throughout Finland during the period July 1 1957–June 30 1958 totalling 57 089 (Series 2) Multiple births and diabetic deliveries were omitted.

The symbols used are as follows

y = duration of pregnancy
x = weight of the child
x = crown-heel-length of the child
x = head circumference of the child
x = weight of the mother
y and x and x^2 and $x^2\ x^2$ = the mean values of the quantities y x x^2 and x^2 x^2 in observation groups determined by the data of the mother and the sex of the child.
b = regression coefficient
s = total deviation of y in the above groups
s_0 = remainder of the standard deviation of y in the above groups after the total deviation of y has been reduced by means of the regression model employed.

The structure of the models and the experiments

The first experiment was made with the following model

$$(I)\ y \quad b + b x_1 + b_2 x_2 + b_3 x_3 + b_{11} x^2 + b_{22} x_2^2 + b_{33} x^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x + b \; {}_1 x_1^3 + b_{222} x_2^3 + b_{333} x_3^3$$

The material consisting of 27 522 cases from Department II of Obstetrics and Gynaecology (Series 1) in the years 1950–1960 and 57,089 cases from the whole country (Series 2) in the year July 1 1957 June 30 1958 was divided into groups as shown in Table I

From both series the following cases were excluded stillbirths and deaths within the first postnatal week, children with mal-

Timonen S Uotila, U Lokki, O Kuusisto, P and Vara, P Acta obst. et gynec scandinav 44 [illegible] 965.

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ESTIMATION OF THE PROBABLE DURATION OF PREGNANCY ON THE BASIS OF THE MATURITY OF THE CHILD

A preliminary report

BY

S. TIMONEN U UOTILA, O LOKKI, P KUUSISTO AND P VARA

Tables estimating duration of pregnancy compiled for forensic purposes are principally based on the size of the child (Föllmer and Könninger 1951 Wichmann 1950) or on the height and weight (Linders Engström and Falconer 1964) with head circumference as an additional factor (Hosemann 1952) In these tables the ranges of duration of pregnancy are expressed as functions of the measurements of the child. From the tables "unsmoothed percentages are often obtained, indicating the degree of probability with which a child having the measurements in question is the product of a pregnancy of given length

If only one measurement is used, the method is relatively simple but inaccurate When several measurements are employed various probabilities are obtained and the table giving the highest probability is used. If the results given by the tables are highly divergent the conclusions drawn must be treated with great caution (Engström and Falconer 1964) There is no theoretical justification for using the mean value of various probabilities since there are variations in the correlations of the different indications of maturity of the child with duration of pregnancy

Material

By special questionnaires data were collected concerning 27 522 deliveries in Department II of Obstetrics and Gynaecology of Helsinki University Central Hospital from the years 1950–1960 (Series 1) Furthermore data were collected concerning all deliveries in maternity hospitals throughout Finland during the period July 1 1957–June 30 1958 totalling 57,089 (Series 2) Multiple births and diabetic deliveries were omitted.

The symbols used are as follows

y = duration of pregnancy
x_1 = weight of the child
x_2 = crown-heel-length of the child
x_3 = head circumference of the child
x_4 = weight of the mother
$\bar{y}$ and $\bar{x}_i$ and $\overline{x_i^k}$ and $\overline{x_i^k x_j}$ = the mean values of the quantities y, x_i, x_i^k and $x_i^k x_j$ in observation groups determined by the data of the mother and the sex of the child.
b = regression coefficient
s = total deviation of y in the above groups
s_r = remainder of the standard deviation of y in the above groups after the total deviation of y has been reduced by means of the regression model employed.

The structure of the models and the experiments

The first experiment was made with the following model

$$(I)\ y = b + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{111}x_1^3 + b_{222}x_2^3 + b_{333}x_3^3$$

The material consisting of 27 522 cases from Department II of Obstetrics and Gynaecology (Series 1) in the years 1950–1960 and 57,089 cases from the whole country (Series 2) in the year July 1 1957–June 30 1958 was divided into groups as shown in Table I.

From both series the following cases were excluded stillbirths and deaths within the first postnatal week children with mal

Table I. *Classification of the Material and Symbols for the Different Groups of Parturients.*

Group		Parity	Age of the Mothers (Years)	Marital Status	Series (Helsinki) Boys	Girls	Series (Whole Finland) Boys	Girls
1	Normal	I	≦ 25	Married	2867	2811	5512	5105
2		I	> 5	Married	1396	1319	3115	3693
3		I	≦ 25	*Unmarried*	413	444	449	353
4		I	> 25	Unmarried	125	169	163	183
5		II	≦ 25	Married	1725	1580	2911	2547
6		II	> 25	Married	2165	2066	4322	4204
7		≦ III	≦ 25	Married	616	604	1060	1078
8		≦ III	26-34	Married	2246	2266	6007	5731
9		≦ III	≦ 35	Married	7758	715	3022	2290
10	*Hyper-*	I	≦ 25		{ 891	{ 804	134	128
11	tension	I	≧ 25				96	86
12		II	all		{ 120	{ 107	133	151
13		≦ III	all				326	234
14	Protein-	I	≦ 25		270	231	268	235
15	uria	I	≧ 25		143	113	120	118
16		II III	all		260	261	352	303
17	Hydrops	I	all		55	59		
18		≦ II	all		78	23		
19	Mild pre	I	≦ 25		{ 242	{ 228	2 1	241
20	eclampsia	I	25				159	130
21		II III	all		130	132	3 0	295
22	Severe	I	≦ 25		1 8	89	{ 196	{ 129
23	pre-	I	> 25		68	60		
24	eclampsia	II III	all		9	93	120	112
	Total cases				40 3	3509	29 1	229 8

formations sick children children whose mothers had diabetes or syphilis and those cases in which the measures y x x and x were lacking

The regression coefficients for model (I) were calculated from the data of all groups except the toxæmic mothers in Series 2 and certain groups in Series 1 The coefficients were determined in such a way that individual terms from Series 1 were included consecutively in the regression model (I) in the order in which

they appear in this model. In Series 2 the order of the regression coefficients included in the model was as follows b b_2 b_1 b_1 b b_1 b_{22} b_{n} b_{n} b b_{22} b_{22}.

When the reducing effect of the different terms in model (I) on the total variation of y is evaluated, the following observations are made

The regression coefficients b_1 and b are highly significant.

b and b_{22} are significant in most groups.

The significance of the deviation from zero of the remaining regression coefficients is weak. As a rule these coefficients are insignificant, although in the groups of moderate size some of them attain the level of almost significant. However all these regression coefficients together explain only 1–3 per cent of the deviation of y In practice therefore they are of no value as compared with the influence of x x x_1^2 and x_2^2, which in the different groups account for about 20–30 per cent of the square of the total variation.

In particular it is noteworthy that after the height and weight of the child have been taken into account, the effect of head circumference on the square of y is negligible

The effect on the model of maternal weight and condition of the child

Since the weight of the mother has been found to have a significant influence on the weight of the child a model based on the hypothesis

$$(II)\ y = b + c\,x_1 + c_2 x_2 + c_{11} x_1^2 + c_{22} x_2^2 + c_{12} x_1 x_2$$

was tested in which x and x were assumed to be expressions of the form

$$x \quad x - f(x) \quad x_2 \quad x_2 - g(x)$$

In other words maternal weight (x) was reduced by a function appropriate to the weight and height of the child $(x_1$ and $x_2)$ In this connexion, by slight reductions and omission of certain terms, a model of the form

$$(II')y = b_0 + b_1x_1 + b_2x_2 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{41}x_1^2 + b_{12}x_1x_2 - b_{14}x_1x_4 + b_{24}x_2x_4$$

was tested for consistence with the data

In this experiment group 6 of the material described in point 3 was used (series 1 II parae age > 25 years) The results were as follows

When argument x_4 was used together with x_1 and x_2, the variation of y was reduced by 20 per cent while the combined influence of x_1 x_2 and x_4 on the square sum was 19.8 per cent. Thus the variation of maternal weight which is marked in the group chosen for the test, does not have such an influence in model (II) as to justify incorporation of maternal weight into the model

A test was also made in order to assess how estimation of duration of pregnancy on the basis of the data of the child is influenced when dead and sick children and children with mal formations are included in the material. The series comprised 100 such children and 2135 normal children The remainder of the standard deviation of y was as follows

Only normal children	1 55 weeks
All children	2 67 weeks

Conclusions concerning a useful model

On the basis of the foregoing following statements may be made

In order to keep dispersion moderate it is advisable to omit the children whose condition is abnormal.

The model

$$(III)\, y = \bar{y} + b_1(x_1 - \bar{x}_1) + b\ (x - \bar{x}_2) + b_{11}\ (x_1^2 - \overline{x_1^2}) + b_{22}(x_2^2 - \overline{x_2^2})$$

is quite sufficient.

The other models tested, which were far more complicated, did not yield essentially better results in regard to estimation of the duration of pregnancy

In model (III) b of models (I) and (II) is replaced by the natural mean values of the quantities calculated from the material, i.e. $\bar{y}$ x_1 and $\overline{x_1^2}$. The variation of the means of the different groups is so wide that it was considered legitimate to use the classification employed in Table I.

Note 1. The small size of the children of the unmarried primiparæ and small $\bar{y}$ were accounted for by the fact that the duration of pregnancy was shorter than in the case of the married primiparæ.

Note 2. Certain other investigations (Timonen *et al.* 1963) have shown that in regard to the relation between height and weight of the child there is a difference between primiparæ and multiparæ. Hence these two groups have been treated separately. Furthermore girls and boys have been kept apart owing to the initial slower growth of the former.

Note 3. The toxæmic groups being very small, great caution is indicated in regard to their models. The groups with proteinuria could be united with the normal group in the evaluations of model (III) without any significant risk of error.

Note 4. The difference between the remainder of the standard deviation obtained in the two series is accounted for by the fact that there are slight differences between the different parts of Finland in regard to the relationship between size and weight of the child and duration of pregnancy, as was observed in another study performed on Series 2 of the present investigation (Timonen *et al.* unpublished). Series 2 comprises children from the whole of Finland. Therefore the remainder must be somewhat greater than in the series collected in Helsinki (Table III).

Note 5. The regression coefficients were calculated on the basis of all groups in Series 1 and almost all groups in Series 2, comprising over 3000 cases. From these calculations the remainder presented in Table II is derived. This reflects how accurately the duration of pregnancy can be estimated on the basis of the length and weight of the child.

Table II. *The Standard Deviation (Remainder) of Duration of Pregnancy after Using the Regression Model (III) The Numbers Give the Remainder in Weeks.*

Group	Series (Helsinki) Boys	Girls		Series (Finland) Boys	Girls
1	1.60	1.58		1.79	1.80
	1.60	1.52		1.71	—
3	1.91	1.75		—	—
4	1.61	—		2.0	06
5	1.58	1.61		1.73	1.-8
6	1.55	1.45		1.58	1.62
7	1.69	1.72		1.-9	1.91
8	1.65	1.5-		1.78	1.73
9	1.-0	1.57		1.75	1.8
10–11	1.73	1.53		—	—
12–13	—	1.63	(13)	1.77	—
14	1.75	1.61		—	—
16	1.69	1.62		1.45	1.75
17	1.-5	1.51		—	—
18	1.69	1.88		—	—
19–20	—	2.09		—	—
21	2.40	1.51		—	1.41
22	1.78	1.55		—	—
23	1.45	1.40		—	—
–4	1.65	1.47		—	—

Table III. *Time Limits (±) for Different Confidence Percentages*

Confidence Percentage	67	80	90	95	99	
of nomogram for series 1 (Helsinki)	1 5	15.5	20.0	23.0	3 5	days
of nomogram for series 2 (whole Finland)	12.3	16.5	1 0	24.5	32.5	days

Nomograms for the estimation of duration of pregnancy on the basis of the size and weight of the child

On the basis of regression model (III) nomograms were made (Fig 1) for the calculation of duration of pregnancy on the basis

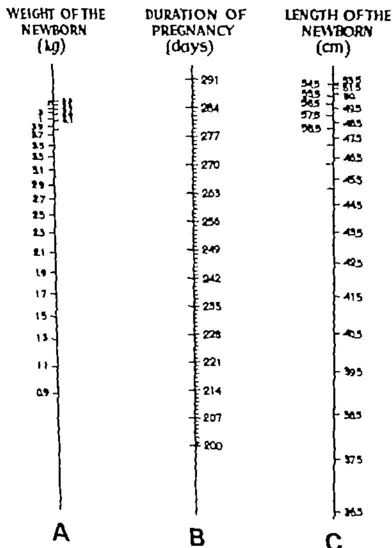


Fig. Nomogram for primiparae girls. Figures for weight (A) and length (C) of the newborn are combined with straight line. The crossing point of thi line and the line B gi es the duration of pregnancy

of the crown-heel-length and weight of the child, for the different groups. In this paper only one example is given of a nomogram from which the duration of pregnancy can be estimated in

the case of primiparæ The instructions for use of the nomogram (Fig 1) are as follows

The nomogram corresponding to the data of the mother is selected

The weight of the child is marked on the weight scale (Fig 1 point A)

The crown heel length of the child (in units of 1 cm) is marked on the size scale (Fig 1 point C.)

A rule is applied to the points marked on the weight and length scales, and the duration of pregnancy in days is read on the duration scale (B)

In order to obtain the limits of confidence for the result according to the criteria indicated in Table III the mean duration of pregnancy obtained from the nomogram must be increased and reduced by the number of days obtained from Table III

If the period under study falls outside the 99 per cent limit, the time of conception as indicated by the result must be regarded as very uncertain (Hosemann)

Example

Age of the mother over 25 years primipara in normal health, *female child length 50 cm weight 3 5 kg, domicile Helsinki.*

Duration of pregnancy (days) obtained from the nomogram 280 5 + 2 5 = 283 days

Duration of pregnancy with 95 per cent limits of confidence 283 ± 23 days.

As was established by Hosemann (1952) the accuracy of *the estimate is to some degree dependent on the maturity* of the child inasmuch as accuracy is reduced in premature deliveries. In practice it is difficult to use different confidence limits for a regression model with more than one parameter In our opinion it is therefore justifiable to use only the limits indicated in Table III which have been obtained in the middle part of the model

Duration of pregnancy from the time of conception (p c)

When duration of pregnancy is calculated for forensic pur poses *individual variations of the menstrual cycle* the possible

occurrence of paracyclic ovulation etc., constitute sources of deviation. In this respect the present study contributes nothing new. In view of what is known concerning the probable time of ovulation, we recommend that the duration of pregnancy as obtained from the nomogram presented above should be reduced by 14 days (*cf.* Stegmann and Hellwig). The difference then indicates the p. c. of the duration of pregnancy.

SUMMARY

A regression analysis for the estimation of duration of pregnancy on the basis of the length, weight and sex of the child and the parity of the mother is described. Data relating to 27 522 deliveries at the Helsinki University Central Hospital and 57,089 at maternity hospitals in the whole of Finland were used. With the aid of the method presented a single value for the probable duration of pregnancy is obtained. Special nomograms designed for practical forensic use are described.

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Duration of pregnancy from the time of conception (p c)

When duration of pregnancy is calculated for forensic purposes individual variations of the menstrual cycle the possible

For these reasons all jaundiced infants in maternity departments are now followed very closely both clinically serologically haematologically and with the help of a range of laboratory tests. They are observed with heightened attention for any signs of kernicterus. Such signs can be grouped into general signs and cerebral signs. Generally the infant is poorly and wretched, perhaps unconscious and with bloody froth around the mouth there may be violent attacks of screaming or merely weak crying, interrupted sleep fever and oedema. The cerebral signs are opisthotonus head retraction increased muscular tone flaccidity clenched fists, head held obliquely tense fontanelle, convulsions, convulsive-like attacks myoclonus, starting, trembling, athetotic like movements of the arms abnormal eye movements and other signs.

In those jaundiced infants both with and without signs of kernicterus during the neonatal period, a variety of more or less serious sequelæ can be recognised later in childhood athetosis plegiæ (especially tetraplegia) impairment of hearing, paralysis of the ocular muscles, hyperkinesia, defects of the dental enamel (especially of the milk teeth) and perhaps behaviour difficulties and inexplicable cases of hyperpyrexia. The complete picture has not yet been completely elucidated.

The mortality rate as well as the incidence of kernicterus and its sequelæ depend both on the ætiology of the clinical jaundice and on its severity and duration From a purely obstetrical point of view it would be interesting to know whether during the course of pregnancy and delivery the obstetrician can make a positive contribution which might benefit the prognosis in infants with icterus neonatorum, and whether he is even able to exert any influence on the incidence of the disease Ignoring in this connection legal interruption of pregnancy on the indication of a known cause of jaundice or counselling against pregnancy in the case of familial incidence of one of these causes.

There are many causes of icterus neonatorum where the obstetrician has no control either of prognosis or of incidence. Fortunately the incidence of such cases is very low for example hereditary spherocytosis lack of glucose-6-phosphate-dehydrogenase hypothyroidism, familial non-haemolytic jaundice (Crig

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ICTERUS NEONATORUM

The obstetrician s influence on its frequency and prognosis

BY

DYRE TROLLE

Icterus neonatorum is taken to mean the presence of clinical jaundice in infants during the first 3–4 weeks of life. Most cases of clinical jaundice are present during the first week of life, more rarely during the second week or later According to Larsen and Wieth (1943) serum bilirubin values less than 8–9 mg per cent cannot be expected to cause clinical jaundice.

Interest in clinical jaundice in the newborn has only come to the fore during the last two decades starting in the period inaugurated by Landsteiner and Weiner s discovery of the Rhesus blood group (1940) It soon became clear that the prognosis in the children of Rhesus-immunized mothers was poor if they did not receive adequate treatment. As a result of lack of treatment, 34 per cent of the infants died neonatally and 5 per cent of the survivors had kernicterus (Vaughan Allen and Diamond 1950)

Later interest concentrated on other newborn infants with clinical jaundice when it became clear that damage due to blood group incompatibility between mother and child could arise from other blood group systems Matters could be just as serious for these infants as for infants of Rhesus-immunized mothers Furthermore it was found that the frequently occurring form of jaundice called physiological jaundice could lead both to death from kernicterus and to survival with sequelæ in the form of cerebral palsy Plum s studies from 1957 1962 and 1964 might be mentioned in this connection

therapeutic usefulness of vitamin K in this age group is minimal indeed. The potential toxicity of such naphthoquinone components in causing hemolysis, hyperbilirubinemia and brain damage has been mentioned previously If for any reason vitamin K is to be used, doses should not exceed 1.0 mg daily

It is difficult to indicate accurately just how great a role is played by sulpha preparations and psychotherapeutic drugs in causing icterus neonatorum. In practise the dilemma can be overcome by avoiding as far as possible the use of these drugs before and during delivery or in medication of the newborn

Infective hepatitis should also be mentioned as a cause of icterus neonatorum. The obstetrician should likewise be alert to the risk of syphilis in the mother Many obstetricians give penicillin treatment to all previous syphilitics early in pregnancy In a case of fresh syphilis or poorly treated syphilis of longer standing, treatment should be instituted as early as possible.

During the actual delivery every intrauterine infection should be treated, just as the newborn infant should in such cases be treated prophylactically by means of antibiotics starting from birth Virus diseases and toxoplasmosis are uncommon and from an obstetrical point of view play a minor role

For the sake of completeness extravascular haemolysis (extensive petechiae or large haematomas) and dehydration should be mentioned The first condition causes jaundice as a result of accelerated haemoglobin destruction at the haemorrhagic sites. The second condition is perhaps caused by circulatory insufficiency

All the above mentioned causes of icterus neonatorum have only a small place in clinical practice The important aetiological factors are first, damage as a result of blood group incompatibility between mother and child, and secondly causes of unknown origin In both these aetiologies the part played by the obstetrician can influence the prognosis and/or incidence of the disease

As far as concerns damage of the child, resulting in *haemolytic disease of the newborn due to blood group incompatibility* it has been found in practice that the three blood groups A, B and D are responsible for 98–99 per cent of the cases of haemolytic

ler Najjar syndrome caused by glucuronyl transferase deficiency) familial benign jaundice (Gilbert's disease) galactosæmia and obstructive jaundice (the latter however does not cause kernicterus)

Recently Arias and Gartner (1964) demonstrated that oral administration of pregnane-3α 20β-diol to newborn infants causes jaundice Earlier they had demonstrated pregnane-3α 20β-diol in the milk from mothers of jaundiced newborn while milk from mothers whose children were normal did not contain it.

The obstetrician may have a certain responsibility for other but likewise rare causes First and foremost come to mind the following drugs vitamin K preparations sulpha preparations and psychotherapeutic drugs since large doses of these drugs, administered to the mother shortly before and/or during delivery can cause hæmolysis in the newborn or disturbances of albumin binding of bilirubin or of bilirubin conjugation The same catastrophe can occur if these substances are administered to the newborn

Vitamin K therapy should undoubtedly be limited to administration to the mother at the commencement of delivery or what is undoubtedly better only to the newborn infant immediately after birth Ten mg of a water soluble preparation of vitamin K can be given to the mother during delivery but there is the risk of overdosage of the fœtus particularly if it is premature (Biskind and Herman 1959) The newborn infant may be given 1–2 mg of the same preparations or of vitamin K intramuscularly (Biskind and Herman) If the dose is to be repeated in the newborn the non toxic vitamin K should be used (Dyggve 1959) It should be mentioned, however that Eastman (1959) has completely given up the routine use of vitamin K and uses it only therapeutically in manifest hæmorrhagic disease of the newborn Reiquam and Smith (1959) go even further "When bleeding is encountered in the immediate neonatal period a cause other than the *normal* low level of prothrombin activity must be sought. Administration of vitamin K will increase the prothrombin activity in the first days of life With recognition of the insignificant role the normally reduced prothrombin activity probably plays in the cause of bleeding in the newborn the

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As far as concerns damage of the child, resulting in *hæmolytic disease of the newborn due to blood group incompatibility* it has been found in practice that the three blood groups A, B and D are responsible for 98–99 per cent of the cases of hæmolytic

disease the A group alone being responsible for 60 per cent (Allen 1959) *Based on the proportions between A and B-affected children in Maternity Department A* the other 40 per cent are found to be distributed as follows B-affected, just under 15 per cent, D-affected just under 25 per cent. The remaining 1–2 per cent are due to other factors within all the blood group systems. In a four year series (1st April 1960–31st March 1964) from Maternity Department A comprising 7926 live born infants weighing over 250 g, the remaining cases were due to the following antibodies anti-c two cases anti-C one case anti-e one case anti E, one case anti M one case anti-S one case and anti JK^b one case *i e 8* cases out of a total of 478 live-born affected infants (1 7 per cent)

Even though hæmolytic disease of the newborn is more often due to the A antigen than to other antigens nevertheless a series of D-immunized mothers will have a higher incidence of infants with hæmolytic disease than a series of A immunized or B-immunized mothers In the above-mentioned four year series from Maternity Department A there were 470 D immunized mothers and 393 affected infants (livebirths + stillbirths), corresponding to 83 6 per cent of the cases (the department is the centre for Rhesus-immunized mothers) while there were 218 A-immunized mothers and 76 affected infants (livebirths + stillbirths) i. e 34 9 per cent. In the case of the B factor corresponding figures were 79 19 and 24 1 per cent. (By A and B-immunization is understood the demonstration of immune antibody the titer of which is at least three dilution steps higher than the titer for complete antibody and by affected condition is understood a positive Munk Andersen dextran/serum conglutination test and/or direct Coombs test) No affected infants were found among the following immunized mothers 41 Lewis immunized 16-Kell immunized 3 Duffy-immunized, 1 Lutheran-immunized and 1 N immunized On the other hand, 5 affected infants were found among 15 mothers who were C-immunized E-immunized c-immunized or e immunized and 1 affected infant was found in each of the following groups 5 M-immunized mothers 1 S-immunized mother and 1 Kidd-immunized mother respectively (P-immunization was not included)

Not only is the infant found to be affected more frequently in the presence of the D-antigen but the course of the disease is also more serious Thus of the 393 D-affected infants 40 died perinatally *i.e.* 10.2 per cent, against 1 death in the 95 A and B-affected infants, *i.e.* 1 per cent. In addition 265 of the 372 liveborn D-affected infants (three infants with hydrops) had to undergo exchange transfusion *i.e.* 71 3 per cent against only 27 6 per cent and 26 3 per cent of the A and B-affected children respectively

Furthermore of the 372 liveborn D-affected infants 324 developed clinical jaundice (the exchange transfusion cases included) *i.e.* 87 1 per cent, while 50 0 per cent of the A-affected and 52 6 per cent of the B-affected developed clinical jaundice.

Even though an unselected series shows only about 0 5 per cent D-affected infants, the importance of the D antigen and the ease with which it can be demonstrated resulted in the decision in Denmark in 1958 that it would be worth while to carry out routine determinations to establish the Rhesus type in all pregnant women and in the Rhesus-negative cases to examine the blood for the presence of anti-D Further all Rhesus-negative women were advised to have a final examination for anti D at about the 35th week. The examinations are free of charge. If anti D is found in the mother's serum, it is recommended that delivery should take place at one of the "Rhesus centres through out the country While these studies are made generally many maternity departments also carry out routine studies for other antibodies as well. It would be reasonable to extend the free Rhesus investigations for pregnant women to include the ABO system for according to the figures quoted above 75 out of every 100 affected infants in an unselected series will be either A or B-affected, while the remainder are D-affected, and it will be necessary to carry out exchange transfusion in 20 of the A or B-affected infants against 17 of the D-affected infants.

There is as yet no recognized method of inactivating the antibodies or of protecting the foetus against them (Lewis, 1964) Liley (1963) and Duggan and Taylor (1964) however have used intraperitoneal transfusion of the intrauterine foetus in one case each, and Freda and Adamsons 1964 have carried out

exchange transfusion in a 27 week-old fœtus *in utero* by per forming laparotomy on the mother and then drawing a leg of the fœtus forward through a uterine incision The patient went into labour on the second day following the operation and gave birth to an infant weighing 800 g which died shortly afterwards

In Maternity Department A we consider it reasonable to induce labour when the fœtus is at a stage when premature birth in itself does not increase the mortality This means that as a routine we induce labour 2–3 weeks before term. If the calculation is uncertain labour is induced at an estimated fœtal weight of approx 2800 g With a rapid rise in antibody titer and/or previous deliveries of severely affected infants with repeated exchange transfusions we have been inclined increasingly during the past year or two to induce labour (or perform a Caesarean operation) 3–4 weeks prior to term

The results from Maternity Departments A and B for the five year period 1954–1959 covering 432 consecutive single births in D immunized mothers have already been published (Lundwall Sorensen and Trolle 1960) The material originates mainly from a period prior to the routine antibody examination of all pregnant women In many of the pregnancies, the treatment has been along the lines laid down above but not a few of the infants were delivered spontaneously elsewhere and only admitted to our departments when jaundice was already evident. The perinatal mortality was 14 6 per cent.

Of the 326 liveborn affected infants (—5 hydropic infants) 16 died during the first week of life *i e* 4 9 per cent. Four of these were not born in the department however but were transferred untreated and moribund during the third to the seventh day of life while a further two had lethal malformations. The corrected neonatal mortality was thus 3 1 per cent A total of 249 infants underwent exchange transfusion *i e* 76 5 per cent. The children

We have carried out the same procedure on one occasion in Maternity Department A, on a 32-week-old fœtus. Here too the patient went into labour spontaneously and ga e birth to a typical hydropic fœtus weighing 1500 g, which died a few minutes later It may well be that such a hydropic fœtus is already out of therapeutic reach early in the second half of pregnancy

were not followed up but were all apparently well on discharge from hospital. According to Vaughan Allen and Diamond (1950) it might be expected that without prophylaxis and treatment, 34 per cent of the liveborn infants would die and 5 per cent would survive with kernicterus.

It is important to examine whether in selected cases an earlier induction of labour in our patients might have reduced the incidence of macerated infants (a total of 31) and hydropic infants (a total of 5) Only 20 of the macerated infants weighed over 2000 g, and it is probable that 11 of them had died 6 weeks or more before term 6 of them 4 to 5 weeks before term, and 3 of them 2 to 3 weeks before term. The 5 hydropic infants were born 6 3 2 2 and 1 week before term.

If these infants at exceptional risk are to be discovered in time other procedures than the ones described above are necessary It is not enough merely to induce labour as a regular procedure for example 4–6 weeks before term as in such a case far too many infants would die neonatally for that reason alone (an estimated 10 per cent) In our five year series from 1954–59, the neonatal mortality following induction was examined for the two weight groups > 2000–≤ 2500 g and over 2500 g (hydropic infants were omitted) Of the 28 infants in the weight group > 2000–≤ 2500 g, the percentage dying was 14.3 per cent, while among the 353 infants in the weight group over 2500 g, the percentage dying was 1 4 per cent. The weight difference was not due to the small infants being the more severe cases but due to erroneous evaluation of foetal size

It is possible on the other hand, that the routine use of percutaneous amniocentesis in the 32nd to 33rd week, with determination f the bilirubin content in the amniotic fluid, may be of some guidance An in estigation of this kind is being conducted at present in Maternity Department A, determining the unconjugated bilirubin content in micromols As yet it is too early to estimate the value of the method and the results in the literature are inconsistent Further the fact must be allowed for that amniocentesis involves certain risks the placenta is exposed to injury with the possibility of foetal erythrocytes being transferred to the maternal circulation resulting in a further rise in the



Fig 1 A portion of the fœtal side of the placenta. A vein and artery are seen in the middle The arrow points to the arterial lesion resulting from percutaneous amniocentesis.

mother s antibody titer In Maternity Department A, the mother s blood is examined for fœtal erythrocytes before and 30 minutes after the puncture by means of Kleihauer s test, but the test was at all times negative as it was also in those cases where pure blood was found on aspiration (Jensen and Sörensen 1964) On the other hand Zipursky Pollack Chown and Israels (1963) have demonstrated the transfer of fœtal blood to the maternal circulation The question whether amniocentesis can in this way provoke a rise in antibody titer or exacerbate the course of the hæmolytic disease thus remains open Fairweather and Walker (1964) found that in some mothers, fever and shivering developed during the hours following the puncture

Recently Queenan and Adams in some cases found as well transfer of fœtal blood to the maternal circulation as a rise in the mother s titre (Obst. et Gyn. 24 530 1964)

They considered that this could be due to the transfer of amniotic fluid to the maternal circulation. In one case these authors injured a hydropic foetus. Further the risk of infection or of lesion of the mother's intestine cannot be completely ignored. It may be added that amniocentesis failed in about 10 per cent of the cases. Finally it might be noted that in one case in Department A, we caused a lesion in an artery on the foetal surface of the placenta with the result that the foetus bled into the amniotic fluid, the latter being heavily bloodstained. The child showed a degree of anaemia much greater than anticipated from his general condition and the lesion on the placenta was recognized macroscopically (Fig. 1)

At present it may be said that the place of amniocentesis in prophylaxis is not yet clarified.

A further possible means of finding infants at special risk is to perform a routine determination of hormone excretion in the urine during pregnancy Taylor Hassner Bruns and Drose (1963) have shown in six cases that oestriol excretion was greater than normal if the foetus was affected and alive, although in a seventh case it lay considerably below the normal level of excretion (no details of this last case are provided) Our impression is that oestriol excretion lies on the average in the upper part of the normal range or above this, but if the foetus is severely affected, the excretion of oestriol is considerably lower than normal. Our series is however too small to allow definite conclusions

Some studies by Connon (1964) on the excretion of chorionic gonadotrophin in the urine suggest that there is a *fall* in the excretion of gonadotrophin during the second trimester followed in the last trimester by a rise to the upper level of normal or over this in cases where the foetus is severely affected. The author herself reports that his communication is only a preliminary one McCarthy and Pennington (1964) have found the maternal HCG serum level to be enormously increased in mothers whose infants were hydropic The level was within normal limits in all other degrees of haemolytic disease The authors suggested that the elevated HCG levels in the maternal serum in hydrops foetalis do not occur before the development of this condition

The obstetrician may also be able *to make a prophylactic contribution* to the outcome of later pregnancies It is in fact possible that a certain number of mothers are immunized in the third stage of labour *Prophylaxis against this is however still uncertain* Some workers recommend that the placenta should be delivered spontaneously, as manipulations with the uterus—Credé expression manual removal—may destroy the barrier between the foetal and the maternal circulations with the result that foetal blood can enter the maternal circulation (*e g* Zipursky Pollack Chown and Israels 1963 Queenan and Nakamoto 1964 and Lewis 1964) Other investigators advise the very opposite namely to remove the placenta manually immediately after birth (Clark and Jacobs 1964) Immunization can also occur by version induction of labour and Cesarean section and perhaps also in toxæmic subjects (Knox Murray and Walker 1961)

The majority of cases of icterus neonatorum are of unknown ætiology In the previously mentioned four year material from Maternity Department A, *jaundice of unknown ætiology* (serum bilirubin 10 mg per cent or more determined by the micromethod of Jendrassik and Groof) was found in 1530 out of 7926 *liveborn infants weighing over 250 g, i. e 19 3 per* cent. (In the author s previously published papers for reasons therein stated the frequency is calculated for single born non-affected infants who survived the first week of life) During the first two years of the study all the newborn received a prophylactic injection of 2 mg menadione intramuscularly immediately after birth the amount being reduced to 1 mg during the last two years of the period No difference in the incidence of jaundice was observed as a result It should be emphasized particularly that the incidence of blood group O among these jaundiced infants was examined during the first two years of the period The value found was 45 per cent *i e* the same as in the normal population Hæmolytic disease was found in only 478 liveborn infants *i e* 6 per cent of the 7926 liveborn *Of these 478 affected infants* only 380 developed clinical jaundice (serum bilirubin 10 mg per cent or more) *i e* 4 8 per cent of all liveborn (D-affected 4 1 per cent,

A-affected 0.5 per cent, B-affected 0.1 per cent and affected against other blood groups below 0.1 per cent)

Jaundice of unknown aetiology is a disease lacking in positive criteria. It is a diagnosis made with a greater or lesser degree of probability after other aetiologies have been eliminated. This disease was previously called physiological icterus but for the time being it is more correct to call it jaundice of unknown aetiology. It is possible that the disease is actually a sign which covers a group of hitherto non-elucidated diseases. Perhaps Arias et al.'s discovery of pregnane-3α 20β-diol in mothers milk will be a guide.

There are certain surmises regarding the aetiology of jaundice of unknown aetiology (*e.g.* Brodersen 1964). For example bilirubin conjugation in the liver may be inadequate as a result of lack of the necessary enzymes (glucuronyl transferase, uridine diphosphate-glucose dehydrogenase and others) co-enzymes and the pre-stages of these (*e.g.* orotic acid). It is also possible that the formation of enzymes and co-enzymes is in itself adequate but fails because the production of bilirubin is abnormally great, or because the catalysts are engaged in other functions (*e.g.* the conjugation of various drugs). A further possible manner in which jaundice might develop is that the bilirubin already conjugated becomes deconjugated in the intestine and resorbed from here, returned to the liver and reconjugated again here, then excreted anew. If a considerable proportion of the bilirubin is resorbed in this manner it will overload the conjugating mechanism and contribute to the development of jaundice. A third possibility is the reduction in the binding of bilirubin to albumin because there is not sufficient albumin available (both intra- and extra-vascularly). The reason may be once again a real lack of albumin or unavailability because the albumin has been taken up by other substances (*e.g.* ketone bodies, unesterified fatty acids, sulphonamides). There are undoubtedly other possibilities.

Whatever may be the causes of jaundice of unknown aetiology clinical experience shows that the frequency of jaundice increases with decreasing foetal weight, *cf.* fig. 2. Foetal weight is however no sure expression for gestational age. In order to determine whether gestational age plays a role, the infants in the weight

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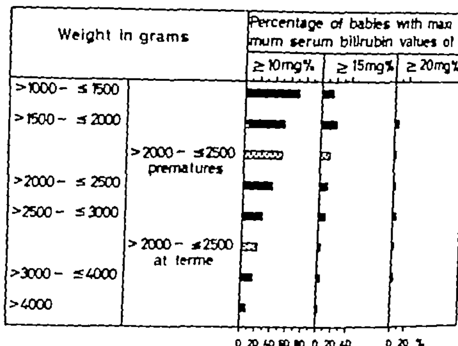


Fig 2 Frequency of jaundice of unknown origin in infants surviving the first week of life. In the weight column, the left half shows the infants grouped according to weight, independent of gestational age. In the right half of the weight column, infants weighing $>2000–\leq 2500$ g are grouped according to gestational age. The serum bilirubin columns show the percentages numbers of infants whose maximum bilirubin values were 10 mg % or over 15 mg % or over and 20 mg % or over respectively

group $>2000–\leq 2500$ g were divided into two groups namely premature infants and underweight but full-term infants. It can be seen clearly that gestational age has an influence on the incidence of jaundice

It has been pointed out in two previous studies (1961 and 1964) that far from all jaundiced infants develop kernicterus or sequelæ in the form of athetosis Based on a study of the literature (especially Plum s investigations 1957 1962 and 1964) and certain calculations the conclusion reached was that exchange transfusion was indicated in 1–2 out of 1000 liveborn infants developing jaundice of unknown ætiology and weighing 2500 g or less at birth and in 0–1 out of 10 000 liveborn infants developing jaundice of unknown ætiology and weighing over 2500 g at

birth. The problem is to find these few infants among the many at an early stage, and this problem is so far unsolved.

On the other hand, what the obstetrician can do today to help solve this problem is to try with all the means at his command to reduce the number of premature, for considered as a group these infants are more than ten times as exposed as mature infants being more exposed the more premature they are The number of liveborn infants of 2500 g or under in Denmark in 1960 was 4212 against 71 534 liveborn infants weighing over 2500 g, i. e 5.6 per cent (*Medico-statistical Reports* 1963) After deducting neonatal deaths and allowing for the incidence of jaundice in the two weight groups, premature and mature infants are seen to contribute equally to the annual number of infants with athetosis following jaundice of unknown origin—the premature group if anything, contributing most.

Precautions against premature births can be divided into arrangements prior to pregnancy and arrangements during the course of the pregnancy Generally the precautions put into operation prior to pregnancy will include myomectomies plastic operations on the uterus because of malformations or cervical insufficiency more rarely a raising of the general health and the institution of adequate therapy for example with regard to malnutrition anaemia, endocrine disease, pulmonary tuberculosis syphilis, cardiovascular disease and infections of the urinary tract. It is likewise advisable that a period of 1 ½ to 2 years should elapse between pregnancies.

During pregnancy there should be careful health control of the mother diet should be adequate, suitable rest should be taken excesses of any kind should be avoided, whether they concern work tobacco alcohol coitus sport or travel. If there has previously been premature birth, the risk of further premature birth is increased, and will be almost 50 per cent with three premature births previously (Bishop 1964) Age likewise plays a role the frequency of prematures being least for the 20–35 year-old mother Diseases accompanying pregnancy but not caused by it should receive careful treatment, as many of them contribute to prematurity Any complication due to pregnancy should be treated as soon as possible, even though the treatment

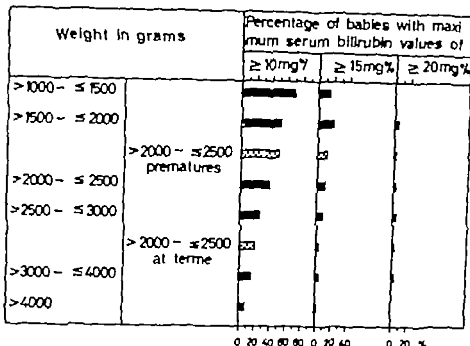


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is often only symptomatic (toxaemia anaemia urinary tract infection other infections bleeding, twins polyhydramnios) Where there has been a previous premature birth as a result of cervical insufficiency, the mother s condition should be closely followed and treatment instituted if necessary The woman s constitution also plays a part, for example women with a small heart volume have an increased frequency of premature births, particularly if they are also anaemic (Räihä, 1962)

In addition to the causes mentioned there are many others which result in premature birth (foetal malformations must particularly be kept in mind) but nevertheless the cause is unknown in up to half the cases In about one fifth of the cases the premature birth *is initiated by spontaneous rupture of the membranes without any obvious reason for this* (Speert, 1964)

Precautions against premature birth must depend on the individual case If causal therapy can be instituted this is obviously best, but it is often necessary to be satisfied with symptomatic treatment such as adequate diet, rest, prohibition of work tobacco coitus sport travelling, etc and continual encouragement of the pregnant woman Not rarely admission to hospital for some months will be necessary Bleeding is treated conservatively as far as possible but repeated blood transfusions are often necessary

In all women with a disposition to premature delivery or with threatening premature delivery the aim is of course to gain enough respite for the infant to be born mature It must not be forgotten however that if premature birth is initiated in spite of all precautions even a few weeks respite will have made it possible to gain much with respect to a reduction in both perinatal mortality and frequency of jaundice Naturally conservative treatment must never be extended at the expense of the health and far less the life of the pregnant woman

SUMMARY

Between one fifth and one quarter of all liveborn infants develop icterus neonatorum (serum bilirubin 10 mg or over) during the neonatal period. A brief account is provided of the

ætiology of Icterus neonatorum and on the basis of the author's material it is shown that the two main causes are jaundice due to blood group incompatibility between mother and infant, and jaundice of unknown origin.

In an unselected series of newborn infants approximately 2 per cent will develop jaundice as a result of blood group incompatibility This incompatibility is within the ABO system in about 75 per cent of the cases within the Rh system in about 25 per cent of the cases and within other blood group systems in 1–2 per cent of the cases.

Further in an unselected series of newborn infants, just under 20 per cent will develop jaundice of unknown origin. The incidence is correlated with gestational age, as the shorter the gestational age, the greater the number of jaundiced infants.

A discussion is provided of the possible ante-natal contribution by the obstetrician in influencing both the prognosis and the incidence of *icterus neonatorum*.

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Addendum

The serum bilirubin values in this study and in two previous studies (1961 and 1964) were determined by the micromethod of Jendrassik and Groof at the Central Laboratory of the Rigs hospital

It has come to my knowledge that readers of the two studies already published have been surprised at the many high values and with good reason The Central Laboratory has recently informed me that a systematical error of 20 per cent is to be found in all values given Originally the factor of 5 32 used for determining the bilirubin concentration in serum was stated by T K With (Nord Med. 13 721 1942) and has since then been used at all Danish and most other Scandinavian laboratories. In order to make the values correspond with the values obtained by the international standard of bilirubin a reduction of 20 per cent must take place In the present study all serum bilirubin values have been reduced accordingly

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FAMILIAL OCCURRENCE OF COEXISTING LEIOMYOMA OF VULVA AND ŒSOPHAGUS

BY

TORE WAHLÉN AND BIRGER ÅSTEDT

Leiomyoma of the vulva is very rare and only 4 cases are on record (Alfieri, 1908 Goldschmied, 1922 Geipel, 1954 Gatter reported by Labhard 1955) Familial occurrence of leiomyoma has never been reported.

Leimyoma of the œsophagus is fairly uncommon Plachta (1962) was able to trace 105 cases in the literature and added 51 of his own (total 156 cases)

He found leiomyomas accounted for 36 per cent of all benign tumours of the œsophagus. Wachsmuth (1959) reported 4 cases of leiomyoma of the œsophagus in members of a single family

At the Department of Obstetrics and Gynæcology Hälsingborg General Hospital leiomyoma of the vulva and coexisting leiomyoma of the œsophagus were seen in a mother and in her daughter

Case A 50-year-old woman with three children, son and two daughters The menopause occurred at 51 years of age. There was no known hereditary tendency to tumour formation The patient had since childhood had difficulty with swallowing and often regurgitated what she had swallowed in 946, when 41 years old, tumour was discovered in the lower œsophagus near the cardia Later that year the patient was subjected to operation (Prof H. Wulff Malmö) with transpleural removal of the tumour which was twice the size of fist and enclosed the tortuous œsophagus. Pathologist report. leiomyoma (Fig.)

After the operation the patient developed diaphragmatic hernia and later recurrent ulcerations of the œsophagus, for which she spent several periods in hospital. At re-operation in 962 the lower part of the œsophagus



Fig. 1. Case. 1. Œsophageal myoma built up of elongated cells interspersed with abundant fibrillar tissue. Htx-eosin. × 190.

was resected because of the widespread ulceration. No residual leiomyoma could be detected.

In 1938 when the patient had been admitted to the Department of Obstetrics and Gynæcology, Hälsingborg General Hospital, because of spontaneous abortion, *hypertrophy of the clitoris* was observed and a *small nodule* was palpated *in the left labium majus*. No treatment had been given. In 1958 the patient returned because the hypertrophy of the clitoris had become more conspicuous since the onset of the menopause two years previously. *She had not noticed any growth of the nodule in the left labium majus.* Examination in 1958 included determination of the urinary excretion of 17-ketosteroids which was found to be normal. Operation was offered but refused.

In 1963 the woman was examined more thoroughly. Body hair growth was *normal and there was no adiposity. The clitoris was enlarged*, about the size of the terminal phalanx of the thumb. Three nodules the size of hazelnuts were palpated in the left labium majus. The nodules were not tender to palpation. Gynæcological examination revealed nothing else of interest.

On determination of the urinary steroids the 17-OH-cortico-steroid-fraction *was found to be somewhat increased*, 12.4 mg/24 hrs. The excretion of 17 ketosteroids was normal. The number of eosinophils and the electrolytes were normal. *The blood pressure was 85/115. Renal function tests* gave normal values. Electrophoresis showed nothing remarkable. LDH-isoenzymes were normal.

Operation showed a number of hazelnut sized fairly firm, yellow-grey lobulated tumours in the left labium majus and in the base of the clitoris as



Fig 2. Case 1 Myoma from left labium majus is fairly rich in cells but shows a tendency to hyalinisation. Htx-eosin. × 90.

well as in the region between the clitoris and the urethra and extending over to the right side Pathologist report. *Leiomyoma* without signs of malignancy (Fig.)

Case 2. A -year-old nulligravida, daughter of the woman in Case The patient elder sister had never had any of the symptoms described below and clinical examination revealed nothing of interest Since the age of 5 years the patient had difficulty in swallowing—a feeling of cramp in the oesophagus and of food tending to be lodged there. She had to eat very slowly to avoid these symptoms In 1963, when 19, tumour of the oesophagus situated just above the cardia was diagnosed At subsequent operation (Prof H Wulff Malmo) large mantle-shaped tumour extending from the cardia to level cm oral thereof was enucleated. Pathologist report *leiomyoma of he oesophagus*

Two years later the patient, who was then 2 sought medical advice because of unusually large external genitalia. Examination revealed *moderate hypertrophy of the clitoris and lump the size of a pigeon egg in the anterior part of he right labium and somewhat smaller lump in the left labium* (Fig 3) No other gynaecological abnormalities were observed.

The patient body build and body hair growth were normal. At laparoscopy the ovaries and other internal genital organs appeared normal. FSH > < 40 I U Urinary oestrogen level determined according to Brown was normal B M R (two estimations)— 2 % and—2 %. P B.I. and serum cholesterol normal. Repeated determination of the urinary steroids showed the 17-OH-corticosteroids to border the upper limit of the normal range. The total eosinophils and serum electrolyte values were normal. The blood pressure was



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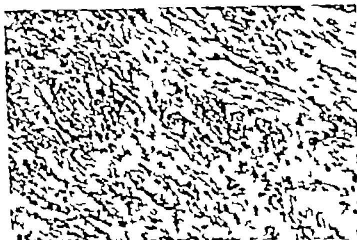


Fig. 4. Case 2. Myoma from right labium majus is built up of criss-cross bundles and rows of elongated cells. Hæ-eosin. × 90.

previous enlargement had thus been due mainly to protrusion of the clitoris by the tumour

As only few cases of leiomyoma of the vulva have been described, and some of them but briefly any practical clinical or theoretical considerations cannot be more than speculative.

Alfieri (1908) desribed a small leiomyoma of the labium majus, which recurred several times. He assumed that it originated from the lowest part of the round ligament.

Goldschmied (1922) reported a plum-sized tumour situated in the left labium majus and originally thought to be a cyst of Bartholin's gland. At operation however a solid tumour was enucleated and pathological examination showed leiomyoma without signs of malignancy No definite connection with the round ligament could be demonstrated.

Gatter (reported by Labhard in 1955) observed a hazelnut sized leiomyoma close to the lower part of the urethra.

Geipel (1954) reported a cherrystone-sized tumour in the right labium minus, below the urethral orifice The tumour was not adherent to the readily movable surrounding tissue and was enucleated without difficulty The tumour a histologically proved



Fig. 3. Case 2. Hypertrophy of clitoris and anterior parts of labia.

140/95. Urea clearance and serum electrophoretic pattern were normal. The shape of the oral glucose tolerance curve was normal. Examination of the ocular fundi and roentgen examination of the sella turcica revealed nothing remarkable.

Operation showed a fairly firm, elastic, somewhat lobulated tumour the size of a pigeon's egg in the right labium and a smaller tumour in the left. The two tumours were connected by a bridge of tumour tissue with a portion the size of a cherry between the clitoris and the urethra. All the tumour tissue was excised. Histological examination showed that the tumour of the vulva was composed of exactly the same type of tissue as the tumour in the mother. Pathologist's report: *leiomyoma* without any signs of malignancy (Fig. 4). After the operation the clitoris was only slightly enlarged and the

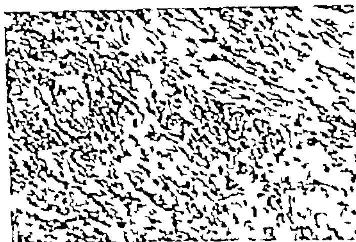


Fig. 4. Case 2. Myoma from right labium majus is built up of criss-cross bundles and rows of elongated cells. Htx-eosin. × 90.

previous enlargement had thus been due mainly to protrusion of the clitoris by the tumour.

As only few cases of leiomyoma of the vulva have been described, and some of them but briefly, any practical clinical or theoretical considerations cannot be more than speculative.

Alfieri (1908) described a small leiomyoma of the labium majus, which recurred several times. He assumed that it originated from the lowest part of the round ligament.

Goldschmied (1922) reported a plum-sized tumour situated in the left labium majus and originally thought to be a cyst of Bartholin's gland. At operation, however, a solid tumour was enucleated and pathological examination showed leiomyoma without signs of malignancy. No definite connection with the round ligament could be demonstrated.

Gatter (reported by Labhard in 1953) observed a hazelnut-sized leiomyoma close to the lower part of the urethra.

Geipel (1954) reported a cherrystone-sized tumour in the right labium minus, below the urethral orifice. The tumour was not adherent to the readily movable surrounding tissue and was enucleated without difficulty. The tumour, a histologically proved

leiomyoma was not demonstrably connected with the urethra, the round ligament or the Bartholin s gland.

From a practical clinical point of view leiomyoma of the vulva is mainly of diagnostic interest. These tumours are rare and can therefore readily be confused with inflammatory conditions of Bartholin s glands But the patient s history and the firmness of the lump should be sufficient for the examiner to suspect a neoplasm

An interesting feature of our cases was that both patients sought advice for enlargement of the clitoris which they felt embarrassing Therefore in both cases an androgen producing tumour of the vulva was suspected. But the patient s habitus and her history were against all known forms of hermaphroditism and pseudohermaphroditism Endocrinological examination laparoscopy and chromosome analysis revealed nothing remarkable The operative findings indicated that the enlargement of the clitoris was due to growth of the tumour in the base of the clitoris

Judging from the cases referred to above a finding of a tumour of the vulva indicates roentgen examination of the oesophagus and, conversely a benign tumour of the oesophagus indicates gynaecological examination

Treatment consists of extirpation Since experience of such tumours is very limited the possibility of their being malignant should be borne in mind and extirpation should, of course be radical. In the cases on record the prognosis has always been good though a tendency to recurrence has been reported (Alfieri 1908) Haemorrhage and haematoma of the labia can disturb the later postoperative course

These tumours are also of theoretical interest. Their origin is obscure Histologically they do not differ from other leiomyomas In two of the cases in the literature (Alfieri, 1908 Goldschmied, 1922) the neoplasms might have originated from the lower part of the round ligament. Sjövall (1955) in his investigation of vaginal myomas thought that some of these tumours had grown from the smooth muscle of the ureter This may also hold for leiomyomas of the vulva and might very well have been the case in our patients though some connection with the end fibres of the round ligament is just as likely or even more probable

The coexistence of leiomyoma of the vulva and of the oesophagus in mother and daughter is noteworthy As expected, chromosome analysis revealed nothing remarkable. There was probably a specific gene complex.

SUMMARY

Leiomyomas of the vulva are extremely rare No hereditary factor has been observed. Leiomyoma of the oesophagus is fairly uncommon. Heredity has ben described. Leiomyoma of the vulva and of the oesophagus in the same patient has never before been reported. Coexisting leiomyomas of the vulva and of the oesophagus in both mother and daughter are described and a specific gene complex is assumed.

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PROGESTERONE METABOLISM IN AN ANENCEPHALIC NEWBORN

I. Metabolites in the plasma

BY

J ZANDER, K. HOLZMANN, AND L. PH. BENGTSSON

During pregnancy considerable amounts of placental progesterone enter the maternal as well as the foetal organism (Zander and v Münstermann 1954 Zander 1959 1961) It was established by previous *in vivo* investigations that placental progesterone is extensively metabolized by the human foetus at relatively early phases of gestation and at the end of pregnancy (Zander 1961 1962 Runnebaum and Zander 1962) Data from Zander and Solth (1953) suggest that the metabolism of progesterone in the newborn is different from that in the adult.

In order to obtain more detailed information on progesterone metabolism in the newborn an anencephalic newborn was perfused with progesterone-4 C and the radioactive material present in the circulating blood and in the tissues was investigated. In this paper data are presented on the circulating radioactive material during and following the perfusion The data on the analysis of the radioactive material in the tissues will be reported in a second paper by Holzmann and Zander (unpublished results 1965)

Methods

Perfusion with progesterone 4- C

The subject was an anencephalic infant born at term. The length of the child was 46 cm, and the weight 2900 g. The progesterone-4 C (specific activity 26.2μc/μmol) was obtained from the Radiochemical Centre Amersham. Its purity was controlled by paper chromatography The perfusion was started 5 hours after delivery 300 ml heparinized blood containing 2.97μc progesterone-4 C in 10 ml ethanol were infused over a period of 60 min. via the umbilical vein. During this period and a subsequent period of 100 min 340 ml blood was collected from the umbilical arteries. Data indicating the time volume relationship for the infusion and collection of blood are summarized in *Table I* The infant died 4 hours after the end of the perfusion.

Extraction and Purification

The blood (samples 1–7 see *Table I*) was separated into red cell and plasma fractions by centrifugation The plasma was

Table I *Infusion of Blood Containing Progesterone-4-¹⁴C in the Umbilical Vein of the Anencephalic Newborn and Collection of Blood Samples from the Umbilical Arteries*

Time (Min)	Blood Infused ml	Blood Infused cpm	Blood Collected ml	Blood Collected cpm	Sample Number
0–	30	90 × 10⁴			
6– 8			50		
9– 4	90	50 × 10³			
5– 9			50	6 × 10⁴	2
20– 26	50	50 × 10³			
23– 29			50	6 × 10³	3
30– 37	50	50 × 10⁴			
3 38			50	1.3 × 10³	4
30– 47	40	50 × 10³			
40– 48			50	18 × 10³	5
49– 60	70	× 10³			
5 59			50	17.2 × 10³	6
99–104			20	9.3 × 10³	7

Values too low due to losses of the solvent during the extraction procedure

deproteinized by adding 5 vol of a mixture of ethanol-ether (3 1) The extracts were cleared by centrifugation (20 min at 800 g) and the precipitates were washed twice with half of the original vol of ethanol-ether (3 1)

The volume of each extract was then reduced to about 5 ml *in vacuo* 40 ml n acetate buffer pH 5 0 was added and the mixture was extracted with chloroform (3 times 40 ml) The chloroform extracts were washed with 1/4 vol n-acetate buffer pH 5.0 and evaporated to dryness 30 ml of water was added and the aqueous mixture extracted thrice with the same vol. of ethylacetate The ethylacetate extracts were evaporated to dryness under reduced pressure and redissolved in 70 % methanol This mixture was allowed to stand overnight at—15 C and centrifuged to remove non steroidal lipids (Zander and Simmer 1954) These purified extracts were designated as *Fraction I* and regarded as containing the free steroids

The aqueous residues containing the conjugate fraction were combined and the volume reduced *in vacuo* to about 40 ml This mixture was incubated at 37 C for 36 hours at pH 5 0 with 300 U/ml Ketodase (Warner Chilcott) to hydrolyse glucosiduronates present. The mixture was extracted with ether (3 × 1 vol.) and the combined ether extracts evaporated to dryness *in vacuo* These extracts were designated as *Fraction II* and regarded as containing the steroidal glucosiduronates

To free further conjugates the aqueous residue was then incubated with 100 U/ml *Helix pomatia* (L'Industrie Biologique Française) at 37 for 24 hours at pH 5.0 The incubation mixture was extracted with ethylacetate (3 × 1 vol) and evaporated to dryness These purified extracts were designated as *Fraction III* and regarded as containing the steroidal sulphates

Paper chromatography

The free and conjugated fractions were subjected to paper chromatography in the following systems

1) hexane methanol water (100 65 35)
2) ligroin benzene methanol water (80 20 80 20)
3) heptan formamide (100 100)

4) ligroin toluene methanol water (50 50 70 30)
5) benzene methanol water (100 50 50)
6) ligroin acetic acid water (100 90 10)
7) cyclohexan ethyl-acetate (100 100)

Measurement of radioactivity

During the fractionation procedure the distribution of the radioactive material was measured by plating aliquots of the different phases and measuring the radioactivity with a thin end-window gasflow proportionality counter (Frieseke and Hoepfner FH 407 and FH 49) The same counter attached to a paper scanning device (Frieseke and Hoepfner FH 452) was used for detecting the radioactivity on the paper chromatograms and for quantitative estimations

Methods of identification

For the identification of the steroids the carrier technique described by Berliner and Salhanick (1956) and Wiest et al. (1959) was used. The localization of U V absorbing steroids on paper chromatograms was performed by contact photography at 254 nm. For the localization of non U V absorbing steroids the following colour reactions were used 1) 10 % phosphomolybdic acid in ethanol, 2) 0 3 % iodine in 5 % KI in water

For the separation of 5α and 5β-derivatives according to Thijssen and Zander (1965) thin layer chromatography was used. Zones of radioactivity on the glass plates were localized by direct scanning of the glass plates with a modified Frieseke and Hoepfner strip scanner The sulphuric acid reaction was used to visualise the carrier steroids.

For the identification of the single compounds the following microchemical reactions were used

) Acetylation carried out with acetic anhydride in anhydrous pyridine for 18 hours at room temperature
) Hydrolysis of acetates with Acylase I (Schering, Berlin) following the technique described by Borgstede et al (963)

3) Oxidation with CrO_3 in acetic acid following the technique described by Borgstede et al. (1963)
4) Reduction or oxidation with the crystallized 20β-hydroxy steroid dehydrogenase of Hübener et al. (1959) following the technique described by Henning and Zander (1962)
5) Oxidation with 3α-hydroxysteroid dehydrogenase prepared according to Koide (1963) following the technique described by Thijssen and Zander (1965)

The identity of the radioactive compounds with a mobility similar to pregnanediol (5ξ-pregnane-3ξ,20ξ-diol) and to pregnanolone (3ξ-hydroxy-5ξ-pregnane-3-one) in system 1 was established out of the possible isomers of these compounds by the use of 20β-hydroxysteroid dehydrogenase and 3α-hydroxy steroid dehydrogenase which are capable of changing specific groups in the molecule and by using thin-layer chromatography as described by Thijssen and Zander (1965)

Results

Fractionation and Identification

The fractionation of Fraction I containing the radioactive material extractable with chloroform, has been summarized in Fig. 1. This fraction was first chromatographed in system 1 and four distinct radioactive zones were detected. These were designated fractions IA–ID.

Fraction IA did not move in system 1 nor in the more polar systems 2, 4 and 5. Thijssen and Zander (1965) have demonstrated that part of the conjugated progesterone metabolites is extracted with chloroform when the extraction follows the deproteinization of the plasma. In view of these findings hydrolysis of the pooled fractions IA from blood samples 1–7 was carried out. The radioactive material did move and separate into two fractions (IA-1 and IA-2) in system 1 when the mixture was rechromatographed after hydrolysis with β-glucuronidase.

Fraction IB separated into two distinct radioactive zones on chromatography in the more polar system 2. These were designated fractions IB-1 and IB-2.

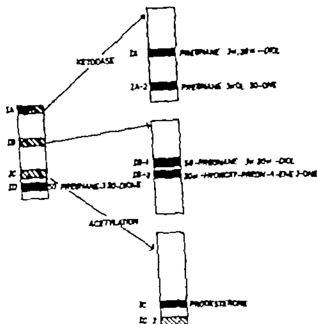


Fig. 1. Fractionation of the chloroform extracts of the deproteinized plasma. For paper chromatography system (hexane methanol water—100 65 35) was used.

Fraction IC showing the mobility of progesterone in system 1 separated into two radioactive zones (IC 1 and IC-2) when the mixture was rechromatographed after acetylation.

The fractionation of the radioactive material in the aqueous phase containing the conjugated steroids is summarized in Fig. 2

After hydrolysis with β-glucuronidase no radioactivity or free steroids could be detected in the ether extract (fraction II)

The sulphate fraction III obtained after further hydrolysis of the water phase with *Helix pomatia* separated into two radioactive zones (IIIA and IIIB) when chromatographed in system 1 After enzymatic oxidation of fraction IIIB with 20β-hydroxy steroid dehydrogenase two radioactive zones were obtained in system 1 fraction IIIB-1 showing the unchanged mobility of fraction IIIB and, in small quantities, a less polar fraction IIIB-2.

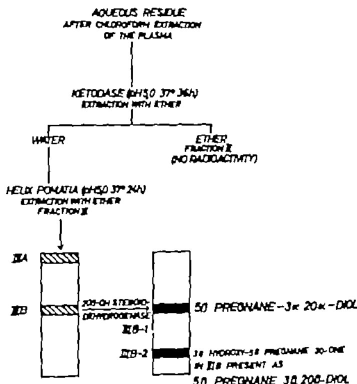


Fig. 2 Fractionation of the water phase after chloroform extraction of the deproteinized plasma For paper chromatography system 1 (hexane methanol water—100 65 35) was used.

The identification of the single subfractions of fraction I and III was based on the following criteria

Fraction IA 1 pregnane 3α,20α-diol (obtained after hydrolysis with Ketodase) This fraction showed the mobility of authentic pregnane 3α 20α-diol in system 1 It could not be oxidized with 20β-hydroxysteroid dehydrogenase Oxidation with 3α hydroxy steroid dehydrogenase resulted in a less polar compound with the mobility of 20α hydroxy-pregnane 3-one The small amounts of radioactivity did not permit us to decide whether the 5α or the 5β isomer was present

Fraction IA 2 3α-hydroxy pregnane 20-one (obtained after hydrolysis with Ketodase) This fraction showed the mobility of authentic 3α hydroxy pregnane 20-one in system 1 Oxidation with 3α-hydroxysteroid dehydrogenase resulted in the formation

of a less polar compound with the mobility of pregnane-3,20-dione. The small amounts of radioactivity did not permit us to decide whether the 5α- or 5β-isomer was present.

Fraction IB-1 5β-pregnane-3α,20α-diol. This substance showed the mobility of authentic pregnane 3α,20α-diol in system 1 It could not be oxidized with 20β-hydroxysteroid dehydrogenase. Oxidation with 3α-hydroxysteroid dehydrogenase resulted in the formation of a compound showing the mobility of 20α-hydroxy pregnane-3-one. The substance obtained was separated from the authentic 5α-isomere by thin layer chromatography and showed the mobility of 20β-hydroxy-5β-pregnane-3-one

Fraction IB-2 20α-hydroxy pregn 4-ene-3-one The identification of this fraction rests on the following criteria 1) Identical mobility with authentic 20α-hydroxy-pregn-4-ene-3-one in systems 1 and 2 2) On acetylation the radioactive material was transformed quantitatively to a compound with the mobility of the acetylated authentic substance. 3) Following hydrolysis with acylase the compound could not be oxidized with 20β-hydroxy steroid dehydrogenase. 4) On treatment with CrO the radioactive material was transformed quantitatively to a compound with the mobility of authentic progesterone. 5) Reduction of this material with 20β-hydroxysteroid dehydrogenase resulted in the formation of a more polar compound with the mobility of 20β-hydroxy-pregn 4-ene-3-one in systems 1 and 2.

Fraction IC 1 progesterone This substance which was not acetylated by treatment with acetic anhydride showed the mobility of authentic progesterone. In system 1 a clear separation between the radioactive compound and pregnane 3,20-dione was obtained. Following reduction with 20β-hydroxysteroid dehydrogenase the radioactive material showed the mobility of 20β-hydroxy-pregn 4-ene-3-one. On acetylation this radioactive material was transformed to a compound with the mobility of the acetylated authentic 20β-hydroxy-pregn-4-ene-3-one.

Fraction IC 2 not identified. After hydrolysis of this acetylated fraction with acylase the radioactive material showed the mobility of a 3-hydroxy-pregnane-3-one in system 1 The amount was too small for further characterisation

Fraction ID 5β-pregnane-3,20-dione The radioactive material

showed the mobility of authentic 5β-pregnane 3 20-dione in systems 1 and 3 It could be clearly separated from progesterone in system 1 It was refractive to treatment with acetic anhydride but on reaction with 20β-hydroxysteroid dehydrogenase formed a substance with the mobility of authentic 20β-hydroxy 5β-pregnane-3-one in systems 1 3 and 6 In the latter system the substance obtained separated from the authentic 5α-isomer

Fraction IIIA not identified This fraction did not move in system 4 nor in the more polar system 5 even after a second hydrolysis with *Helix pomatia* The amounts were too low for identification

Fraction IIIB 1 5β pregnane 3α,20α -diol (obtained after hydrolysis with *Helix pomatia*) This fraction which could not be oxidized by 20β-hydroxysteroid dehydrogenase showed the mobility of authentic pregnane 3α 20α-diol The identification was based on the criteria used for the identification of fraction IA 2. In addition fraction IIIB-1 was oxidized with CrO to a compound showing the mobility of pregnane-3α 20α-dione. Reduction of this substance with 20β-hydroxysteroid dehydrogenase led to a compound with the mobility of 20β hydroxy 5β-pregnane-3-one. In system 6 the substance obtained separated clearly from the authentic 5α isomer

Fraction IIIB-2 5β pregnane 3β,20β-diol (obtained after hydrolysis with *Helix pomatia*) By enzymatic oxidation of fraction IIIB with 20β-hydroxysteroid dehydrogenase a small part (6 %) of the fraction was converted to a product with the mobility of authentic 3β-hydroxy pregnane 20-one (IIIB 2) indicating that the C-20 hydroxyl group of the substrate was in the 20β-position The product formed with the mobility of 3β-hydroxy pregnane-20-one could not be oxidized with 3α hydroxysteroid dehydrogenase In thin layer chromatography it showed the mobility of the 5β-isomer and separated from the authentic 5α isomer

Quantitative results

The results obtained in determination of the amounts of radioactivity present in various fractions of the samples collected during the perfusion experiment are summarized in Fig 3

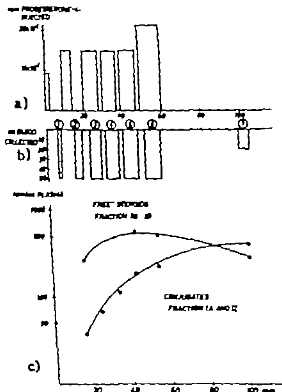


Fig. 3 Schematic representation of the experimental data adopted for the perfusion experiment) cpm/progesterone-4 °C injected per time unit into the umbilical vein. b) ml blood collected per time unit from the umbilical arteries. Samples designated numbers -7) amounts of radioactivity (cpm/ml plasma) in fractions IB–ID ("free steroids) and fraction IA plus III ("conjugated" steroids)

They demonstrate an increase in the free steroids (fractions IB-ID) extracted with chloroform until the end of the infusion of progesterone-4 C and a decrease thereafter During the infusion the amounts of radioactivity present in the conjugated form (glucuronides fraction IA and sulphates fraction III) are seen to be lower than the levels of the free steroids. 40 minutes after the end of the perfusion the level of the conjugates was found to be higher than that of the free steroids.

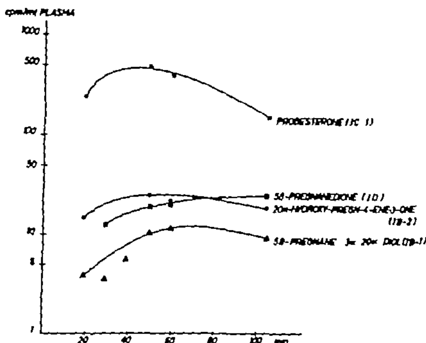


Fig. 4. Levels of the free steroids identified from fractions IB–ID (samples number 2–7) during and after the perfusion of progesterone-4-^{14}C.

Fig 4 represents the quantitative distribution of the free steroids identified from fraction I During the perfusion of progesterone-4 C 80–90 % was found to be due to progesterone After the end of the perfusion experiment the amount of progesterone decreased 40 minutes after the end of the perfusion about 45 per cent of the radioactivity was found to be due to metabolites of progesterone 20α hydroxy pregn 4-ene 3-one 5β pregnane 3α 20α-diol and 5β-pregnane-3 20-dione

Quantitative results for the conjugates during and after the perfusion are summarized in Fig 5 It represents a gradual rise in the amount of radioactivity of the glucosiduronates (fraction IA) and the sulphates (fraction III) It has been pointed out that after hydrolysis more than 90 per cent of the radioactivity in the sulphates was found to be due to 5β-pregnane 3α,20α-diol

The percentile distribution of the free metabolites of progesterone-4 C (fraction IB-1 IB-2 IC-2 and ID) the glucosiduronates (fraction IA 1 and IA 2) and the sulphates (frac

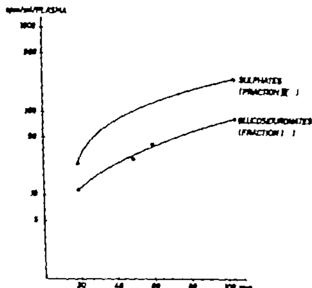


Fig. 5. Levels of the sulphates (fraction IIIB) and the glucosiduronates (fraction IA) (samples number 2–7) during and after the perfusion of progesterone-4-^{14}C

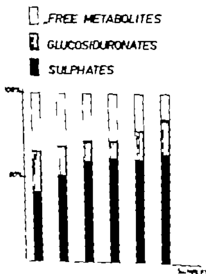


Fig. 6. Percentile distribution of the "free" progesterone metabolites (fractions IB–ID) glucosiduronates (fraction IA) and sulphates (fraction IIIB) in plasma samples 2–7 during and after perfusion of progesterone-4-^{14}C.

tion IIIB 1 and IIIB-2) is demonstrated in Fig 6 It shows an increase in the major part of the progesterone metabolites con jugated as "sulphates" and a decrease in the free" metabolites during and after the perfusion experiment.

Discussion

The results presented in this study demonstrate that progesterone 4 C introduced via the umbilical vein into an anen cephalic newborn is in part metabolized by its tissues. 65–85 per cent of the circulating progesterone metabolites were found to be in a conjugated form The major part of the conjugates was present in the sulphate" fraction "Glucosiduronates counted only for a minor part of the conjugates

These data suggest that progesterone metabolites are extensively sulphurylated by the human anencephalic newborn

Thijssen and Zander (1965) used methods identical to those in this study for extraction hydrolysis purification and separation of progesterone metabolites from plasma after in travenous injection of progesterone 4 C into adult women Nearly all of the radioactivity of the conjugates was found after hydrolysis with β-glucuronidase only very small amounts of radioactivity were detected in the ether extracts after further hydrolysis of the aqueous residue with *Helix pomatia*

It is concluded from the data in this study and those reported by Thijssen and Zander (1965) that the conjugation pattern of progesterone metabolites in the anencephalic newborn appears to differ significantly from that in adult women where the major part of the circulating conjugates appears in the glucosiduronate fraction It has yet to be determined whether conjugation of progesterone metabolites in the normal newborn follows a pattern similar to that in the anecephalic newborn In this connection it is of interest that the newborn period as well as intrauterine life is also characterized by a great ability for sulfurylating circulating oestrogens and that this ability is considerably reduced during adult life (Diczfalusy *et al* 1961 1964 Mikhail *et al* 1963a b Troen *et al* 1961 Wilson *et al* 1964)

The predominant steroid identified in the hydrolyzed sulphate

fraction of the plasma samples of the anencephalic newborn is 5β-pregnane-3α,20α-diol. Beside this compound, a small quantity of 5β-pregnane-3β,20β-diol was identified. The position of the sulfurylation in these steroid molecules is unknown. After hydrolysis of the glucosiduronates 5?-pregnane-3α,20α-diol and 3α-hydroxy-5?-pregnane-20-one were partially identified. The same steroids were identified from the hydrolyzed circulating conjugates after intravenous injection of progesterone-4 C into adult women (Thijssen and Zander 1965) It would also appear from the data given in this study and by Thijssen and Zander (1965) that the qualitative nature of the free progesterone metabolites identified in the plasma samples of the anencephalic newborn does not differ in principle from that of the circulating free progesterone metabolites in adult women.

The quantitative results of the perfusion experiment demonstrate an increase in the circulating free steroids until the end of the infusion of progesterone 4 C and a decrease thereafter However the conjugates show a further increase after the end of the infusion. This may be explained by further metabolism of the circulating progesterone 4 C and conjugation of the metabolites after the end of the infusion.

SUMMARY

An anencephalic newborn was perfused with progesterone 4 C and the circulating radioactivity was analysed. 65–85 per cent of the circulating progesterone metabolites were found to be in a conjugated form. The major part of the conjugates was present in the sulphate fraction Glucosiduronates accounted for only a minor part of the conjugates.

The predominant steroid in the hydrolyzed sulphate fraction was identified as 5β-pregnane 3α,20α-diol. 5β-pregnane-3β,20β-diol could be detected in small quantities. In the free fraction progesterone 20α hydroxy-pregn 4-ene-3-one 5β-pregnane-3α 20α-diol and 5β-pregnane-3 20-dione were identified.

The data suggest that circulating progesterone metabolites are extensively sulphurylated by the human anencephalic newborn and that the conjugation pattern differs significantly from that in adult life

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FŒTAL ERYTHROCYTES IN THE MATERNAL CIRCULATION

BY

HARRY ZILLIACUS

Introduction

Erythrocytes which contain fœtal hæmoglobin can now be distinguished from those containing hæmoglobin of the adult type. This is made possible because of a difference in the composition of the amino acid chain of the globin molecule The fœtal hæmoglobin is more resistant to alkali and acid than the adult hæmoglobin. Alkali denaturation has for a long time been used to estimate the concentration of fœtal hæmoglobin in a solution of a mixture of both the hæmoglobins (Singer Chernoff and Singer 1951 Jonxis and Visser 1956) The identification of fœtal and adult hæmoglobin within erythrocytes was made possible by the staining technique first described by Kleihauer Braun and Betke in 1957 In this procedure the adult hæmoglobin is eluted from the erythrocytes by a citric acid phosphate-buffer During the subsequent staining, erythrocytes containing fœtal hæmoglobin are stained red whereas the erythrocytes from which the hæmoglobin has been eluted appear as unstained ghosts The gradual increase in number of erythrocytes containing adult hæmoglobin the proportion of erythrocytes containing both adult and fœtal hæmoglobin and the change of the ratio of fœtal-adult hæmoglobin during intrauterine development of the fœtus can be closely followed by this technique (Vartiainen 1963) In premature infants the concentration of fœtal hæmoglobin is significantly elevated (Brody and Nilsson, 1960)

Since the recognition of fœto-maternal transfusion by Levine and associates in 1941 by Næslund in 1946 and by Wiener in 1948 this problem has received increasing attention. Chowns description in 1954 of transplacental hæmorrhage from the fœtus to the mother has been followed by numerous reports of fœto-maternal transfusion The Kleihauer staining technique has been utilised by a number of investigators for identification of fœtal cells in the maternal circulation (Oehlert 1963 Clayton, Feldhaus and Whitacre 1964 Zilliacus, 1963, 1964)

The Problem

The aim of the present investigation was to assess the frequency with which fœtal cells are found in the maternal circulation before during and after delivery in both normal and abnormal obstetrical circumstances. In an earlier survey (Zilliacus *et al* 1961) fœtal erythrocytes were found in the material circulation in 49 3 per cent cases with various obstetric abnormalities. In another survey (Wimhöfer *et al* 1962) fœtal erythrocytes were found in the maternal blood in 39 3 per cent cases following obstetric operations.

Material and Methods

Ninety-seven normal parturients and 59 patients with obstetric abnormalities were selected for examination of fœtal erythrocytes in the maternal circulation Blood samples were obtained from the finger tip for screening of fœtal red cells within 24 hours of delivery Blood samples for antepartum examination were collected 1–14 days before delivery Thirty two of the 97 normal cases and all of the abnormal cases were examined for the occurrence of fœtal erythrocytes before delivery as well

The blood samples were fixed and stained according to the technique described by Kleihauer and Betke The slides were searched throughout for the presence of fœtal erythrocytes. Control films were made with blood samples obtained from non pregnant gynæcological patients.

Table I. *Foetal Erythrocytes in the Maternal Circulation in Normal and Abnormal Obstetrical Conditions*

	Normal Cases		Abnormal Cases	
	Number of Cases	Positive Smears	Number of Cases	Positive Smears
Antepartum	32		59	6
Postpartum	87	8	59	22
	19	= 7 8 per cent	118	38 = 32.2 per cent

Scores of Foetal Erythrocytes

Normal Cases	Abnormal Cases
= 6	1 = 7
2–4 = 3	2–4 = 13
132 = 1	5– = 0
	1–20 = 4
	30 =
	–4 per mille = 3

Table II. *Foetal Erythrocytes in the Maternal Circulation in Various Abnormal Obstetrical Conditions*

Obstetrical Diagnoses		Number of Foetal Erythrocytes Found Antepartum	Postpartum
Premature separation of the placenta or bleeding	(4)	6	8
Late toxaemia	(5)	4	7
Anaemia	(5)	—	3
Diabetes	( )	—	
Breech presentation	( )		
Polyhydramnios	( )		—
Others	(20)	4	
		16	22

Blood groups of the ABO-system of both the mother and the newborn infant were determined in 74 of the normal and 24 of the abnormal cases.

Results

A study of all cases both ante and post-partum revealed that 19 6 per cent smears contained foetal erythrocytes. The results

Table III. *Foetal Erythrocytes in Maternal Circulation in Relation to Foetal and Maternal ABO Blood Group Systems*

	Mother	O	O	O	O	AB	AB	AB	AB	A	A	A	A	B	B	B	B	Total
	Foetus	O	AB	A	B	O	AB	A	B	O	AB	A	B	O	AB	A	B	
Normal cases	Positi e	0	1	1	0	0	1	0	1	1	2	0	0	1	0	0	0	8
	Negative	8	6	5	1	0	2	1	2	3	2	17	1	5	4	1	8	66
Abnormal cases	Positive	1	0	1	1	0	0	0	2	4	0	5	1	1	0	0	0	16
	Negative		0	2	2	0	0	0	0	0	1	1	0	0	0	0	1	8
																		98

Positive Incompatible	Normal	5
	Abnormal	5
Positive Compatible	Normal	3
	Abnormal	11

are summarised in Table I. There were 10 positive slides among the 129 samples obtained from normal cases which gives a frequency of 7.8 per cent. *The corresponding figures among the* abnormal cases were 38 positive slides out of 118 a frequency of 32.2 per cent. In both groups there were more "positive" smears in samples obtained post-partum than ante-partum.

Among the normal patients 1 to 4 foetal erythrocytes were found in 9 cases. In one sample collected after delivery 132 foetal erythrocytes were found on the slide. In this case only one foetal erythrocyte was found in the film made before labour started. The patient was a gravida 9, para 7. The blood group of both mother and child was AB. The newborn infant, weighing 3,930 gr was healthy and was not anaemic.

Among the cases with obstetric abnormalities foetal scores ranged from 1 foetal erythrocyte to 4 per mille of erythrocytes. Foetal erythrocytes were found most frequently in the maternal circulation in cases of premature separation of the placenta and in late toxaemia. In one of these a case of premature separation of the placenta, there were 2 per mille foetal erythrocytes before delivery and 4 per mille after delivery (blood groups mother AB infant B). In another case of premature separation of the placenta, 3 foetal erythrocytes were found on the slide before delivery and 1 per mille post-partum (blood groups mother 0, infant 0).

ABO groups of mother and foetus were determined in 8 of the 10 normal cases with positive smears 5 showed ABO incompatibility and 3 were compatible. Among 16 abnormal cases with positive smears similarly examined foeto-maternal ABO-incompatibility was found in 5 and compatibility in 11.

Discussion

The overall finding of 19.6 per cent positive smears in patients investigated differs from results obtained by other workers. Wimhöfer *et al.* who observed 765 cases *reported* an incidence of 10.6 per cent foeto-maternal bleeding after normal deliveries and 39.3 per cent after various surgical obstetrical procedures. Clayton *et al.* found 20 per cent positive slides in the ante-partum period with a peak of 47 per cent at term.

Table III *Foetal Erythrocytes in Maternal Circulation in Relation to Foetal and Maternal ABO Blood Group Systems*

	Mother	O	O	O	O	AB	AB	AB	AB	A	A	A	A	B	B	B	B	
	Poetus	O	AB	A	B	O	AB	A	B	O	AB	A	B	O	AB	A	B	Total
Normal cases	Positive	0	1	1	0	0	1	0	1	1	2	0	0	1	0	0	0	8
	Negative	8	6	5	1	0	2	1	2	3	2	17	1	5	4	1	8	66
Abnormal cases	Positive	1	0	1	1	0	0	0	2	4	0	5	1	1	0	0	0	16
	Negative	1	0	2	2	0	0	0	0	0	1	1	0	0	0	0	1	8
																		98

Positive Incompatible	Normal	5
	Abnormal	5
Positive Compatible	Normal	3
	Abnormal	11

circulation in case of premature separation of the placenta and in late toxaemia.

ABO groups of mother and foetus were determined in 8 of the 10 normal cases with positive smears 5 showed ABO incompatibility and 3 were compatible. Among 16 abnormal cases with positive smears similarly examined foeto-maternal ABO incompatibility was found in 5, and compatibility in 11

I wish to thank Mrs. A.-M. Ottelin for her skilful technical assistance.

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In the present series the frequency of "positive" slides in normal (7 8 per cent) and abnormal (32.2 per cent) obstetric cases was markedly different. Foetal erythrocytes occurred 4 to 5 times more frequently in the abnormal cases "Positive" slides were found after delivery in the normal series 4 times and, in the abnormal series 1.4 times more frequently than before the delivery These observations indicate that uterine muscular activity which affects the thin cell-layers separating the foetal and maternal circulation and pathological changes in the placenta are the chief factors facilitating the passage of foetal erythrocytes into the maternal circulation The relatively small difference in number of "positive slides before and after delivery in the abnormal cases points to the same possible cause of the foeto-maternal haemorrhage Increasing uterine activity as term approaches is likely to result in damage to the foeto-maternal cell barrier particularly if it has been weakened by some pathological process such as toxaemia

The detection of foetal cells in the maternal blood in 10 cases of ABO incompatibility can be explained by variation in the strength of the antibody titres present. In a previous observation of a case of foeto-maternal ABO incompatibility it was noted that agglutinated and disintegrating foetal erythrocytes disappeared from the mother s blood over the course of two days (Zilliacus, 1963)

SUMMARY

The Kleihauer Betke technique for staining of erythrocytes which contain predominantly foetal haemoglobin was applied to blood smears otbained from the fingertip of 97 normal parturients and 59 abnormal cases

Overall 19 6 per cent slides contained foetal erythrocytes.

Among the normal obstetrical cases foetal erythrocytes occurred in the material circulation in 7 8 per cent. In cases with obstetrical abnormalities there was a four times higher incidence of positive slides (32.2 per cent) In both groups there were more positive smears in samples obtained post partum than in those obtained ante-partum

Foetal erythrocytes were found most frequently in the maternal

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ILLARY FILTRATION DURING NORMAL PREGNANCY

BY

SVEN SPETZ

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In 1932 Krogh, Landis and Turner introduced the pressure plethysmograph in studying capillary filtration rate. By this technique the blood vessels in a tissue are collapsed to measure

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CAPILLARY FILTRATION DURING NORMAL PREGNANCY

BY

SVEN SPETZ

In an earlier paper forearm blood flow during normal pregnancy was studied (Spetz, 1964). As regards cellular nutrition however volume blood flow through a tissue is of less importance than the manner in which blood is distributed within the tissue. The function of the peripheral blood circulation is *inter alia* to supply nutritients to the tissues and to remove metabolites. Although some molecules such as water diffuse freely between plasma and tissue, net movement of fluid takes place mainly by capillary filtration. There are important differences between these two modes of transfer. The diffusion is extremely rapid, the filtration slow and provoked in response to transcapillary pressure disequilibrium (Starling, 1896, Landis, 1934).

The rate and direction of the transcapillary filtration are dependent upon the relation between the hydrostatic and the colloid osmotic pressure gradients acting across the capillary membranes and on the porosity of the capillary wall (Starling, 1896). On the arterial side of the capillaries hydrostatic pressure tends to filter fluid from the blood. On the venous side of the capillaries fluid returns to the blood due to the colloid osmotic pressure of the plasma proteins. Normally there is an approximate balance between the hydrostatic and colloid osmotic forces acting across the capillary membranes.

In 1932 Krogh, Landis, and Turner introduced the pressure plethysmograph in studying capillary filtration rate. By this technique the blood vessels in a tissue are collapsed to measure

small increments of tissue volume produced by filtered fluid. This method has been used among others by Krogh, Landis, and Turner (1932) Landis and Gibbon (1933) McLennan (1943 1946) Kitchin (1954) in studying capillary filtration in man and animals

Pappenheimer and Soto Rivera in 1948 developed a gravimetric method which proved very useful for studying capillary filtration in experimental animals

During normal pregnancy capillary filtration rate has been measured by McLennan (1943 1946) From these studies it was evident that the filtration rate increased towards the end of pregnancy

In 1960 Mellander described another volumetric method to study capillary filtration. The method has been used among others by Folkow and Mellander (1960) Lewis and Mellander (1962) Celander and Mårild (1962) Cobbold, Folkow Kjellmer and Mellander (1963) Kjellmer and Odelram (1963) Ablad and Mellander (1963) Jacobsson and Kjellmer (1964) Mellander Öberg, and Odelram (1964) and Öberg (1964) to study capillary filtration in animals and also in the feet of newborn infants and of adults

The present paper deals with the capillary filtration rate in the human forearm during normal pregnancy using a modification of the volumetric method described by Mellander (1960)

Material

Twenty non-pregnant women were used as controls. Their ages varied between 16 and 34 years None of the subjects had been pregnant. Each of them was studied once only and under same experimental conditions as the pregnant group

Twenty-one pregnant women were studied. Their ages varied between 20 and 40 years Sixteen subjects were nulliparous 5 women had had a previous normal pregnancy and delivery The subjects were followed with repeated examinations at intervals of 2 to 3 months In total 55 separate examinations could be performed on the pregnant group

Methods

Theoretical considerations. At rest the forearm of a subject has a constant volume. The arterial inflow of blood to the forearm and the venous outflow are of the same magnitude. The filtration on the arterial side of the capillary bed is as great as the return of fluid on the venous side (Starling equilibrium) By the sudden inflation of a sphygmomanometer cuff on the upper arm venous outflow is hindered and the venous pressure in the distal region increased. The increase in venous outflow pressure will be followed by an accumulation of blood in the regional venous reservoirs When the pressure within the veins becomes higher than the occluding pressure blood will pass the sphygmomanometer cuff As long as the cuff pressure is less than the diastolic blood pressure blood flow in the forearm will be little interfered with (Conrad and Green 1961) The increase in venous outflow pressure is transmitted backwards to the capillaries so that the mean capillary hydrostatic pressure is raised. The equilibrium of hydrostatic and colloid osmotic forces will be altered so that a net filtration of fluid takes place into the extravascular space After a rapid increase in forearm volume due to the arterial inflow of blood there will be a slow continuous increase in forearm volume due to capillary filtration These two phases in volume increase can be measured with a plethysmograph placed around the forearm. Landis and Gibbon (1933) have proved that there is a linear relationship between filtration rate in the forearm and applied venous pressure. The capillary filtration into the tissues will continue until the tissue hydrostatic pressure has increased enough to counteract outward filtration (Landis and Gibbon 1933 Pappenheimer and Soto Rivera 1948)

General experimental conditions. Measurements of capillary filtration rate were performed simultaneously with measurements of forearm blood flow The experimental conditions thus were identical (Spetz, 1964) Outward filtration was studied at cuff pressures of as a rule, 40 and 60 mm Hg, the pressures being always lower than the diastolic blood pressure. In a distal cuff around the wrist pressure was held high to exclude the circulation to the hand. Care was always taken to ascertain that

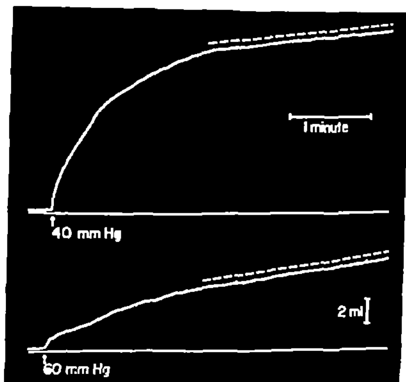


Fig. 1 Filtration curves at cuff pressures of 40 and 60 mm Hg. In this case the CFC was calculated to 0.0044 ml/min. × 100 ml × mm Hg (for further information see text)

the subject was in a comfortable position as discomfort or tension of muscles could render the measurements invalid. Often the patient fell asleep during the examination As some patients were anxious and not satisfactorily relaxed a large number of experiments could not be performed.

Calculation of capillary filtration rate A recording of capillary filtration at two different cuff pressures is seen in Fig 1 The first part of the curve represents forearm volume at rest. After a few seconds the two sphygmomanometer cuffs are inflated, the proximal to 40 mm Hg The rapid increase in forearm volume which follows is due to distension of the veins by the arterial inflow of blood (the normal plethysmogram) After some minutes the increase in volume due to the distension of the veins decreases and blood passes the occluding cuff From the slow but continuous increase in forearm volume which

follows net filtration of fluid across the capillary membranes can be calculated. Thereafter the proximal cuff pressure is increased to 60 mm Hg. A short "inflow phase" is seen, thereafter a steeper filtration slope due to a further increase in capillary hydrostatic pressure.

The capillary filtration rate is usually expressed as capillary filtration coefficient, CFC (Pappenheimer and Soto-Rivera, 1948, Pappenheimer 1953). CFC gives the quantity of fluid passing through the capillary area per unit tissue in unit time, per unit of pressure difference across the capillary membranes. The coefficient is generally expressed in ml/min × 100 ml × mm Hg.

In this study the generally accepted approximation has been adopted that at rest 80 per cent of the total vascular resistance resides in the pre-capillary section (Pappenheimer and Soto-Rivera 1948, Pappenheimer 1953, Renkin and Pappenheimer 1957, Folkow and Mellander 1960, Mellander 1960). This implies that if the venous outflow pressure is increased 20 mm Hg only 16 mm Hg will be transmitted backwards to the capillary section (see *Discussion*).

Statistical analysis. For calculation of arithmetic mean, standard deviation, confidence limits, and regression line conventional statistical methods were applied (Hald, 1951). The significance of differences between groups was tested by the t-test. A significance level of 5 per cent was chosen for the design of the experimental model to be tested by statistical analysis.

The relation between CFC and duration of pregnancy (t) is expressed as a linear regression function

(1) $CFC = \alpha + \beta(t - \bar{t}) \quad 11 \leq t \leq 40$

where β is the coefficient of regression. The constants α and β are calculated from the experimental observations by the method of least squares. To test whether the linear function fits the material the variances have been tested by Bartlett's test and the significance of the linearity by analysis of variance.

Results

In the non-pregnant control group the mean value of CFC was found to be 0.0046 ± 0.0015 ml/min. × 100 ml × mm Hg

Table I

Number	Duration of Pregnancy in Weeks	Cuff Pressure mm Hg	Capillary Filtration Rate ml/min/100 ml	CFC ml/min × 100 ml/mm Hg
1	17	30	0.06	
		50	0.08	0.0013
	25	40	0.09	
		60	0.17	0.0050
2	13	40	0.12	
		60	0.23	0.0069
	18	40	0.10	
		60	0.17	0.0044
	32	40	0.10	
		60	0.19	0.0056
3	10	40	0.11	
		60	0.16	0.0031
	27	40	0.13	
		60	0.25	0.0075
	37	40	0.09	
		60	0.16	0.0044
4	12	30	0.05	
		50	0.12	0.0044
	38	40	0.08	
		60	0.17	0.0056
5	16	30	0.07	
		50	0.13	0.0038
	24	40	0.08	
		60	0.18	0.0063
	32	40	0.14	
		60	0.17	0.0019
	39	40	0.09	
		60	0.19	0.0063
6.	12	40	0.12	
		60	0.17	0.0031
	30	40	0.13	
		60	0.25	0.0075
	37	40	0.10	
		60	0.15	0.0031
7	11	40	0.06	
		60	0.14	0.0050
	22	30	0.08	
		50	0.17	0.0056
	31	40	0.18	
		60	0.26	0.0050
8.	23	30	0.05	

Capillary filtration during normal pregnancy

Number	Duration of Pregnancy in Weeks	Cuff Pressure mm Hg	Capillary Filtration Rate ml/min 100 ml	CFC ml/min. 100 ml mm Hg
			0.10	0.0031
		50	08	
	29	40	0.18	0.0063
		60	07	
	38	40	0.15	0.0050
		60	0.14	
9.	8	40	0.23	0 0056
		60	0.08	
	33	40	0.20	0075
		60	0.15	
0.		40	0.22	0044
		60	07	
	36	40	0.17	0063
		60	0.	
	13	30	0.17	0044
		50	0.04	
	8	40	0.16	0.0075
		60	09	
2.	12	40	0.17	0 0050
		60	3	
	20	40	.17	0088
		60	4	
	28	40	0.24	0063
		60	08	
3.	26	40	0. 9	0069
		60	0.13	
	37	40	0.19	0.0038
		60	3	
4	3	30	20	0044
		50		
	27	40		0056
		60	09	
	36	40	8	0056
		60		
5.	24	40		
		60	0. 5	0.0025
			07	
	32	40	6	0056
		60		
6.	4	30		
		50	8	0044
		40	07	
		60	5	0050
	3	40	07	
		60	8	0069

Number	Duration of Pregnancy in Weeks	Cuff Pressure mm Hg	Capillary Filtration Rate ml/min. × 100 ml	CFC ml/min. × 100 ml × mm Hg
17	20	40	0.06	
		60	0.15	0.0056
	33	40	0.05	
		60	0.17	0.0075
18	20	30	0.12	
		50	0.23	0.0069
	30	30	0.08	
		50	0.13	0.0044
19.	12	30	0.14	
		50	0.21	0.0044
	22	30	0.07	
		50	0.16	0.0056
	29	40	0.10	
		60	0.16	0.0038
	39	40	0.10	
		60	0.15	0.0031
0.	30	40	0.13	
		60	0.20	0.0044
	39	40	0.14	
		60	0.24	0.0063
21	11	40	0.05	
		60	0.16	0.0069
	20	40	0.06	
		60	0.18	0.0075
	27	40	0.10	
		60	0.16	0.0038
	36	40	0.14	
		60	0.19	0.003

All data for the pregnant group concerning filtration rate at different cuff pressures and CFC are seen in Table I

As seen from Table I CFC differs in the same subject from one determination to another but no constant trend could be found. A mean value of all observations was 0.0052 ± 0.0016 ml/min. × 100 ml × mm Hg The difference between this value and that for the control group is not statistically significant ($t = 1.46$ df = 73)

In order to evaluate any possible change in CFC during pregnancy the gestation period (t) has been divided in three-week

Table II

Duration of Pregnancy in Weeks	Number of Observations	Mean Value of CFC ± Standard Deviation of the Value
1 13	8	0050 ± 00 3
14– 6		0.0041 ± 0003
7– 9	3	0.0044 ± 0.0025
20–22	8	0.0062 ± 0.0012
23–25	5	0 0043 ± 0012
26–28	6	0.0060 ± 0 0009
29–3	7	0055 ± 0.00 3
32–34	5	0056 ± 0.0020
35 37	6	0044 ± 0.0012
38–40	5	0053 ± .0012
Total	55	0051 ± 00 6

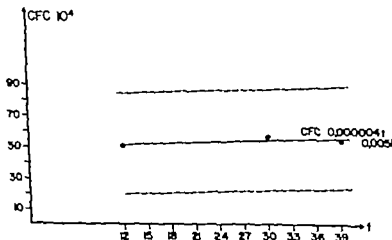


Fig 2. CFC × 10^4 as function of the duration of pregnancy (solid line) and the 95 per cent confidence limits for single value of CFC × 10^4 (dotted lines)

periods. The mean values calculated on the basis of all observations made in each three-week-period are seen in Table II.

As seen from Table II there is no tendency for the CFC-values to change in any direction.

Table III

Source of Variation	Sum of Square σ^2	Degree of Freedom	Mean Square σ^2	F	Critical Value $F_{.05}$
Slope of line	63 14	1	161 69	0.27	4.05
Variation of means of true groups about the line	3232.39	8	404.05	1 73	2.15
Variation within groups	10480.00	45	232.89		
Total	13775 53	54			

The relation between CFC and t (Fig 2) was calculated from the experimental observations

CFC=0.000004 t + 0 0051 according to (1 1)

The variances of CFC for the different periods of pregnancy were tested and found to be independent of the period

An analysis of variance for the regression function between CFC and t is seen in Table III

The analysis of variance for linearity permits the conclusion that the functional relation corresponds to the assumed model [$F \approx 1\ 73$ $df \approx (8\ 45)$] The 95 per cent confidence limits for β were $-0\ 00005 - +0\ 00005$ From Table III it is obvious that the regression coefficient (slope of line) does not statistically differ from 0

The CFC values have been calculated for the total forearm tissue mass Volume changes can occur only in tissues outside the skeleton As the skeleton constitutes only about 6 per cent of total forearm tissue volume (calculation based on volume measurements of radius and ulna on female skeletons) no corrections of the values have been performed.

During pregnancy there may thus be a small increase in CFC (Fig 2) but this increase is not statistically significant.

Discussion

CFC differs widely in different tissues. There are two factors in particular which may influence the CFC value for a special tissue. One factor is the size of the capillary surface area avail-

able for filtration exchange. The other factor is the porosity of the capillary membranes (Pappenheimer 1953)

The capillary surface area available for filtration exchange is dependent upon the number of capillaries actually open to blood flow at any given time. The number of capillaries open for filtration is dependent on the tone of the pre-capillary sphincters By opening of previously closed capillary channels an increase in the rate of filtration is to be expected. It is obvious that neurohormonal factors not only control the hemodynamics of the resistance and the capacitance vessels per se but that they also have an influence on the filtration exchange between the extravascular and intravascular spaces by influence on the tone of the pre-capillary sphincters (Renkin and Pappenheimer 1957 Folkow and Mellander 1960 Mellander 1960 Landis and Pappenheimer 1963 Cobbold, Folkow Kjellmer and Mellander 1963 Oberg, 1964)

The other factor which will determine CFC for a tissue is the permeability of the capillary membranes In the literature there are conflicting ideas about the capillary permeability during pregnancy McLennan (1943 1946) Szontagh (1949) Burger (1951) Herold and Bräutigam (1955) Pinoli and Russo (1956) and Abel (1960) have given evidence of an increased permeability during pregnancy

According to Poiseuille s law the flow of liquid through a tube or pore (other factors remaining constant) is directly proportional to the fourth power of the diameter of a tube. Even a small change in capillary permeability would result in a considerable increase in CFC The experimental findings presented here indicate no such considerable increase during pregnancy Since it is known (Spetz, 1964) that there is a dilatation of the resistance vessels during pregnancy there is every reason to believe that there is also a similar influence on the pre-capillary sphincters, increasing the size of the capillary surface area available for filtration. The small increase in CFC during pregnancy is therefore more probably due mainly to a change in the size of the capillary surface area and not to an increase of the capillary permeability

Important factors which may influence capillary filtration

during pregnancy should be discussed. One of these is a change in mean capillary hydrostatic pressure

Capillary hydrostatic pressure to a great extent depends on the relationship between the vascular resistance proximal to the capillary level and the resistance distal to it (pre- to post capillary resistance ratio) Whenever the resistances in these two vascular sections are affected, a shift in the ratio may occur resulting in a change of capillary hydrostatic pressure (Kitchin, 1954 Renkin and Pappenheimer 1957 Mellander 1960 Landis and Pappenheimer 1963 Öberg, 1964) Capillary hydrostatic pressure has been measured directly in few tissues In man determinations have been limited to the capillary loops in the nail bed where arterio-venous anastomoses are present and may influence pressure measurements (Landis and Pappenheimer 1963)

The ratio of pre- to post-capillary resistance in man was assumed to be 4 1 Eighty per cent of a given increase in venous outflow pressure is transmitted backwards to the capillaries. One-fifth of a pressure increase at the venous side will therefore be lost before the capillary level is reached. If venous outflow pressure is increased by 20 mm Hg as in the present study only about 16 mm Hg will be transmitted backwards to the capillaries (Pappenheimer and Soto Rivera 1948 Renkin and Pappenheimer 1957 Cobbold Folkow Kjellmer and Mellander 1963) By calculation of CFC the difference in filtration rate between the two cuff pressures has been divided by 16

In animals a sudden vasodilatation leads to a decrease in the ratio between pre- and post-capillary resistance. The mean hydrostatic capillary pressure rises leading to an increase of net outward capillary filtration (Ablad and Mellander 1963 Jacobsson and Kjellmer 1964) During pregnancy there is a decrease in the peripheral resistance to blood flow most evident after the 30th week of gestation (Spetz, 1964) A change in the pre- to post-capillary resistance ratio would result in an increase of net outward capillary filtration lasting for many months provided there are no factors counteracting the outward filtration rate e g a rise of the colloid osmotic pressure Even a

small change in the ratio from 4 : 1 to 3 : 1 will give an increase in capillary hydrostatic pressure of 5 mm Hg, the mean arterial blood pressure being estimated as 100 mm Hg. In a person weighing 60 kilo and with a CFC-value of 0.0050 ml/min. × × 100 ml × mm Hg, outward filtration under such circumstances has been calculated as 2160 ml in 24 hours. Jacobsson and Kjellmer (1964) have shown that the lymph flow is small compared with the rate of net outward capillary filtration. Thus fluid must accumulate outside and inside the fascial boundaries giving an increase in tissue pressure. The increase in tissue pressure counteracts the effect of a raised mean capillary hydrostatic pressure on outward filtration (Landis and Gibbon, 1933 Kitchin 1954). During normal pregnancy there are no signs of an increase in tissue pressure in the forearm. There is no oedema. With a high tissue pressure it would further be impossible to record constant filtration rates for 5 to 10 minutes, as in the actual series.

An increase in the colloid osmotic pressure could counterbalance the rise in the outward filtration rate. However there is a progressive decline in total plasma protein concentration during pregnancy (Lagercrantz, 1945 Scrimshaw and Alling, 1949 Coryell, Beach, Robinson Macy and Mack, 1950 Mack 1960 Reboud, Gronlade Groslambert, and Colomb 1963). There is a decrease in the colloid osmotic pressure parallel to the changes in the concentration of plasma proteins (Dieckmann 1952). A decrease in colloid osmotic pressure results in an additional net loss of fluid by filtration.

Venous pressure in the forearm is constant during pregnancy (Cohen and Thomson 1936 McLennan, 1943).

Due to the water in the plethysmograph the tissue pressure in the forearm must be somewhat increased. A rise in cuff pressure from 40 mm Hg to 60 mm Hg corresponds to an increase in venous outflow pressure of 20 mm Hg, as has been shown by Celander and Mårild (1962). This observation has been confirmed in the present study when the venous pressure in the forearm, surrounded by water in the plethysmograph, was measured in two subjects.

The viscosity of blood decreases during pregnancy due to the

rise in plasma volume and the relative decrease in red blood cell volume (Cohen and Thomson 1939 Kellar 1950 Dieckmann 1952 Vorys, Hanusek and Ullery 1963) Pappenheimer in 1953 proved a relation between the viscosity of blood and capillary filtration rate In the actual study these changes in viscosity are small and seem to be of no quantitative importance

From the discussion above it seems logical to conclude that *although there is a continuous decrease in the peripheral resistance to blood flow during pregnancy the pre- to post-capillary resistance ratio must remain unchanged* However even if this ratio does fall from 4 1 to say 3 1, and some unknown mechanism compensated the resulting increase in outward filtration rate such a change would not invalidate the results presented The increase in CFC during pregnancy is in any case *not statistically significant*.

Capillary filtration during normal pregnancy has been studied by McLennan (1943, 1946) He used pressure plethysmography a procedure in which the circulation in the forearm is interrupted by repeated supra-systolic cuff pressures With the assumption that 80 per cent of an increase in venous outflow pressure is transmitted backwards to the capillaries McLennan (1943 1946) found a CFC-value in non-pregnant women of 0 0037 ml/min × 100 ml × mm Hg This coefficient for the control group is lower than that found by Landis and Gibbon (1933) 0 0057 ml/min × 100 ml × mm Hg, and that in the present study During pregnancy McLennan (1943 1946) found that the CFC-value increased to 0 0050 ml/min × 100 ml × mm Hg The patients were studied only once and during the last 8 weeks of pregnancy The latter value is in close agreement with the mean value of all observations made in the present series. From the increase in CFC McLennan (1943, 1946) drew the conclusion that capillary permeability must increase during pregnancy However the discussion above shows that *it is impossible to draw such a conclusion Other factors e g* an increase in capillary surface area, must be discussed The volumetric method used in the present study interferes little with the blood circulation through the tissues and therefore appears

to be a more physiological procedure than pressure plethysmography

SUMMARY

1. Capillary filtration rate in the forearm was studied during normal pregnancy. A volumetric method was used. Twenty one pregnant women were examined as a rule four times during pregnancy and compared with a control group of 20 non-pregnant women.
2. Non-pregnant women had a capillary filtration coefficient of 0.0046 ± 0.0015 ml/min $\times$ 100 ml $\times$ mm Hg. A mean value of 55 examinations during pregnancy was 0.0052 ± 0.0016 ml/min $\times$ 100 ml $\times$ mm Hg. There is no statistically significant difference between these two values. During pregnancy there is a small increase in capillary filtration coefficient but this increase is not statistically significant.
3. Different factors which can influence capillary filtration rate during normal pregnancy are discussed.

Acknowledgements

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PERIPHERAL CIRCULATION IN PREGNANCY COMPLICATED BY TOXÆMIA

BY

SVEN SPETZ

It is a generally accepted idea that hypertension in toxaemia of pregnancy is due mainly to an increase of the peripheral resistance to blood flow caused by constriction of the peripheral vessels. The widespread pathological lesions found in patients who have died of eclampsia are considered to be due to a generalized spasm of peripheral blood vessels (Hunter and Howard, 1961 Willson Beecham, and Carrington 1963 Lewis, 1964) There is, however only a small number of investigations concerning the peripheral vascular bed in toxaemia of pregnancy As regards the blood circulation in muscles and skin an increase in the peripheral vascular resistance has so far not been clearly shown. In fact, it seems to be the contrary The most extensive study of the blood flow in muscles and skin of patients with pre-eclampsia (7 cases) has been published by Burt (1950) The values of blood flow were higher than for normal pregnant women indicating a dilatation of the resistance vessels both in muscles and skin.

Capillary filtration rate in normal pregnancy and in pre-eclampsia has been studied by McLennan (1943a 1946) During normal pregnancy there was an increase in capillary filtration rate which could not be found in toxaemia.

The results presented by Burt (1950) thus are somewhat conflicting and the number of observations few In the present paper therefore, forearm blood flow and capillary filtration rate

were studied in a larger series of patients with toxæmia of pregnancy The results were compared with those obtained for normal pregnancy (Spetz, 1964 Spetz, 1965) In these studies a marked dilatation of the resistance vessels in the muscles and skin was found during normal pregnancy Capillary filtration rate in the forearm did however not show any statistically significant change when compared with non-pregnant women Such a comparison with the situation during normal pregnancy seems to be of importance to obtain further insight into the hæmodynamic situation in pre-eclamptic patients

Material

For the diagnosis of pre-eclampsia the definition and classification given by the American Committee on Maternal Welfare were used Thus all patients by these standards appeared normal during early pregnancy After the 24th week of gestation at least two of the following symptoms had to be found for justifying the diagnosis

1) systolic blood pressure of 140 mm Hg or above and/or diastolic blood pressure of 90 mm Hg or above,
2) albuminuria
3) œdema in the face or hands and/or gain in weight of two kilograms or more in one week due to retention of fluid.

In total 38 women were examined prior to treatment of the pre-eclampsia. Thirty two of the subjects were pregnant for the first time and 6 had been pregnant previously The age range was 17–29 years.

The results were compared with those obtained during normal pregnancy (Spetz, 1964 Spetz, 1965)

Methods

A detailed description of methods and experimental conditions has been published earlier (Spetz 1964 Spetz, 1965) In the present paper therefore, only a short presentation of the methods will be given.

Forearm blood flow

Resting forearm blood flow was determined in the right forearm by venous occlusion plethysmography

Peripheral resistance to blood flow

This was expressed as peripheral resistance units, PRU and calculated by dividing mean arterial blood pressure by resting forearm blood flow (Green Lewis, and Nickerson 1944) Functional mean arterial blood pressure was estimated by adding 1/3 of the pulse pressure to the diastolic pressure. The blood pressure was measured by the conventional sphygmomanometer cuff method.

Maximal blood flow capacity

This was taken as the peak blood flow occurring during a reactive hyperaemia, elicited by 5 minutes arterial occlusion and by muscular work during the last 30 seconds of arterial occlusion.

Capillary filtration rate

This was determined by utilizing a modification of the volumetric method described by Mellander (1960) A net out ward filtration was provoked when hydrostatic capillary pressure was increased by raising the venous outflow pressure with a cuff. The increase in tissue volume due to filtered fluid, was measured with the plethysmograph Capillary filtration rate was measured at two different cuff pressures, as a rule 40 and 60 mm Hg, and expressed as a capillary filtration coefficient, viz CFC, ml fluid filtered per min × 100 ml tissue × mm Hg (Pappenheimer and Soto Rivera, 1948)

Statistical analysis

Statistical analysis was performed in the same manner as in the previous papers A significance level of 5 per cent was selected for testing the differences between groups. For comparison between the regression functions in the normal pregnant series and in the pre-eclamptic group conventional statistical methods were applied (Hald, 1951)

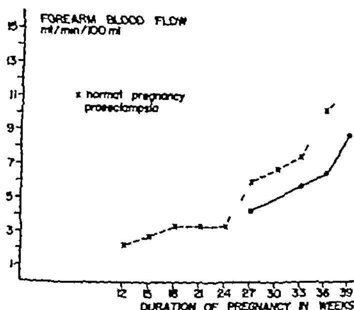


Fig 1 The mean values of forearm blood flow in normal pregnancy (dotted line) and in pre-eclampsia (solid line) in relation to the duration of pregnancy

Table 1

Duration of Pregnancy in Weeks	Number of Observations	Mean of Observed Values of Blood Flow ml/min/100 ml	Mean of the $\log_e$ Values of Blood Flow	Standard Deviation of the $\log_e$ Values
26–28	3	4.2	1.38	0.46
29–31	0	0	0	0
32–34	4	5.8	1.65	0.50
35–37	17	6.5	1.72	0.62
38–40	14	8.7	1.94	0.76

Results

To give a suitable presentation of the results the duration of pregnancy as calculated from the first day of the last period, was divided into three-week periods From all the observations in each period mean values of resting forearm blood flow peripheral vascular resistance maximal blood flow capacity and net capillary filtration rate were calculated.

Resting forearm blood flow

The number of observations in each period, the mean values of

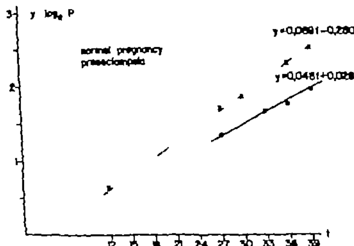


Fig. 2. Forearm blood flow in normal pregnancy (dotted line) and in pre-eclampsia (solid line) as function of the duration of pregnancy (t). Upper equation, normal pregnancy lower equation, pre-eclampsia.

blood flow means of the logarithmic values for blood flow and standard deviation of the logarithmic values are seen in Table I.

Fig 1 shows how the blood flow changes during pregnancy in the normal pregnant series and in the pre-eclamptic group. The data indicate mean values for each three-week-period.

As seen from Fig 1 there is in pre-eclampsia a tendency to increasing blood flow towards the end of the pregnancy The blood flow is however always lower than during normal pregnancy

The relations between the logarithmic mean values for blood flow ($\log_e P$) and the duration of pregnancy (t) in the normal and pre-eclamptic series are seen in Fig. 2.

In pre-eclampsia the following results were found

$y = \log_e P = 0.048 \times t + 0.028$ according to (1 3) or
$P = 1.03 \times e^{0.048 \times}$ according to (1 1) [Spetz (1964)]

The variances in the different periods were tested and found to be independent of the period. An analysis of variance *for linearity* may permit the conclusion that in pre-eclampsia there is a linear functional relation between $\log_e P$ and t [$F = 0.06$ $df = (2\ 34)$] A comparison between the regression functions in the two

Table II

Duration of Pregnancy in Weeks	Number of Observations	Mean of the Observed PRU-values	Mean of the log_e Values of PRU	Standard Deviation of the log_e Values
26–28	3	33	3.40	0.40
29–31	0	0	0	0
32–34	4	30	3.29	0.51
35–37	17	26	3.05	0.61
38–40	14	22	2.82	0.[illegible]4

series gives the result that the functions may be parallel ($t = 0.70$ $df = 126$) The difference between the lines *however is statistically significant* ($t = 3.50$ $df = 127$)

Thus *there seem to be significant differences* in the mean values of forearm blood flow in the two series

Peripheral resistance to blood flow

Table II shows the number of observations in each period, the *mean values for vascular resistance in PRU means of the logarithmic* values for PRU and standard deviation of the logarithmic values

Fig 3 shows how the mean values for PRU change in the normal and pre-eclamptic series The peripheral vascular resistance decreases towards the end of the pregnancy in both groups. However it is evident that the vascular resistance is greater in pre-eclampsia than in normal pregnancy This implies that the tone of the resistance vessels in the forearm is higher in the pre-eclamptic series

Fig 4 depicts the relations between the logarithmic values in the two series and the duration of pregnancy

In pre-eclampsia the following results were obtained

$z = \log_e PRU = -0.054 \times t + 4.968$ according to (1.4) or $PRU = 144.03 \times e^{-0.051 \times t}$ according to (1.2) [Spetz, (1964)]

An analysis of variance for linearity showed a functional relation between log PRU and t [$F = 0.13$ $df = (2\ 34)$] A comparison between the regression functions in the normal and pre-eclamptic series *showed that the functions may be parallel* ($t = -0.40$ $df = 126$) The difference between the lines is statistically significant ($t = -5.82$ $df = 127$)

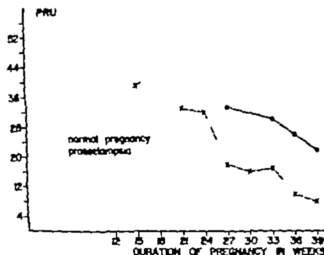


Fig. 3 The mean values of the peripheral vascular resistance to blood flow in normal pregnancy (dotted line) and in pre-eclampsia (solid line) in relation to duration of pregnancy

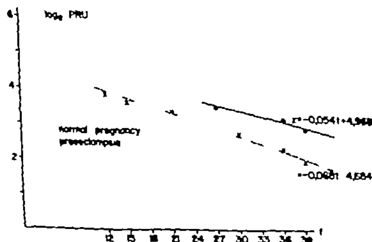


Fig. 4 Peripheral vascular resistance to blood flow in normal pregnancy (dotted line) and in pre-eclampsia (solid line) as function of the duration of pregnancy ( ) Upper equation pre-eclampsia, lower equation: normal pregnancy

Table II

Duration of Pregnancy in Weeks	Number of Observations	Mean of the Observed PRU-values	Mean of the $\log_e$ Values of PRU	Standard Deviation of the $\log_e$ Values
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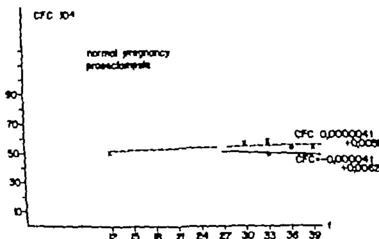


Fig. 5 CFC × 10^4 in normal pregnancy (dotted line) and in pre-eclampsia (solid line) as a function of the duration of pregnancy (*t*) Upper equation: normal pregnancy lower equation, pre-eclampsia.

An analysis of variance for the regression function between CFC and the duration of pregnancy (*t*) gave the same result as for the normal pregnant series [F = 0.68 *df* = (3 17)] The regression coefficient (slope of line) does not differ statistically from 0. A comparison between the regression functions in the two series permits the conclusion that the functions may be parallel (t = 0.30 *df* = 73) Furthermore, the difference between the lines is not statistically significant (*t* = 0.92 *df* = 74) Therefore the regression functions may be identical.

A mean value for all observations (22) in the pre-eclamptic series was 0.0048 ml/min × 100 ml × mm Hg. There is no statistically significant difference between this value and the mean value for all observations made during normal pregnancy 0.0052 ml/min. × 100 ml × mm Hg (*t* = 0.67 *df* = 75)

Discussion

The American Committee on Maternal Welfare has classified cases of pre-eclampsia as mild or severe The classification severe requires at least one of the following symptoms

Table III

Duration of Pregnancy in Weeks	Number of Observations	Mean Value of CFC ± Standard Deviation of the Values
26–28	1	0.0038 ± –
29–31	1	0.0044 ± –
32–34	3	0.0048 ± 0.0015
35–37	11	0.0054 ± 0.0024
38–40	6	0.0038 ± 0.0018
Total	22	0.0048 ± 0.0021

These results do not contradict the assumption that there is a relative constriction of the resistance vessels in patients with pre-eclampsia as compared with normal pregnant women.

"Maximal blood flow capacity"

In the 35th–37th week of gestation the mean value for all observations in pre-eclampsia was 32.2 ml/min × 100 ml, and in the 38th–40th week 38.4 ml/min × 100 ml. The mean values for the peripheral vascular resistance during peak blood flow in these three-week-periods were 4 PRU and 3 PRU respectively.

The corresponding mean values for "maximal blood flow capacity" in normal pregnancy were 32.5 ml/min × 100 ml and 34.8 ml/min. × 100 ml, and for the peripheral vascular resistance 3 PRU in the two groups.

Since the resistance vessels in the two series can dilatate to the same extent the relative constriction of these vessels in the pre-eclamptic group must be due to a higher tone of the vascular smooth muscles and not to a structural change.

Capillary filtration rate

The mean values of CFC calculated for each three-week period can be seen in Table III.

The relation between CFC and the duration of pregnancy (Fig. 5) was calculated from the experimental observations $CFC = -0.00004 \times t + 0.0062$ according to (1.1) [Spetz (1965)]. The variances of CFC in the different periods were found to be independent of the period.

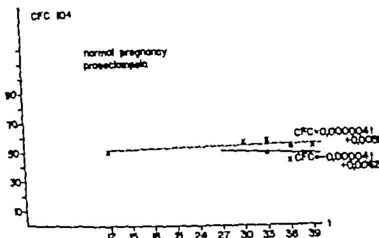


Fig 5. CFC × 10^4 in normal pregnancy (dotted line) and in pre-eclampsia (solid line) as a function of the duration of pregnancy (t) Upper equation, normal pregnancy lower equation, pre-eclampsia

An analysis of variance for the regression function between CFC and the duration of pregnancy (t) gave the same result as for the normal pregnant series [$F = 0.68$ $df = (3 \ 17)$] The regression coefficient (slope of line) does not differ statistically from 0 A comparison between the regression functions in the two series permits the conclusion that the functions may be parallel ($t = 0.30$ $df = 73$) Furthermore the difference between the lines is not statistically significant ($t = 0.92$ $df = 74$) Therefore the regression functions may be identical.

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The American Committee on Maternal Welfare has classified cases of pre-eclampsia as mild or severe The classification severe requires at least one of the following symptoms

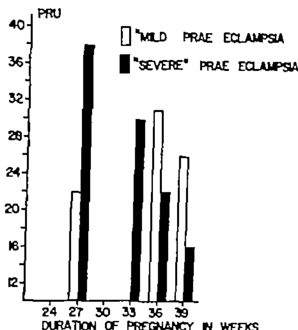


Fig. 6. The mean values for peripheral vascular resistance to blood flow in mild" (white columns) and severe (black columns) pre-eclampsia. For further explanations, see text!

1) systolic blood pressure of 160 mm Hg or above and/or diastolic pressure of 110 mm Hg or above
2) albuminuria of 5 Gm or more in 24 hours
3) oliguria ($\leq$ 400 ml urine in 24 hours)
4) cerebral or visual disturbances
5) oedema of the lungs or cyanosis

In the present series the pre-eclampsia in 17 subjects was classified as mild and in 21 subjects as severe There seems to be no direct correlation between this classification and the degree of relative vasoconstriction As seen from Fig 6 the subjects with mild pre-eclampsia as a rule showed higher values for PRU than the severe cases

The most common lesion of the placenta in pre-eclampsia is a widespread infarction In some subjects there are reasons to believe that the function of the placenta is impaired (Nesbitt 1958 Reid 1962 Budliger 1964 Lewis 1964) A reduction of the oxygen supply to the foetus has been demonstrated in cases associated with placental insufficiency Furthermore the

foetus usually is small and emaciated and fails to grow as pregnancy proceeds. Sometimes the foetus dies *in utero* prior to the onset of labour

In the present series there were 4 still-births. In these cases the signs of pre-eclampsia appeared early between the 28th and the 32nd week of gestation. The mean value of the peripheral vascular resistance for these 4 subjects was 38 PRU This is a high value as compared with the corresponding mean value for normal pregnancy which is 16 PRU (Spetz, 1964)

In 17 subjects the signs of pre-eclampsia appeared between the 35th–37th week of gestation The children in 5 of these cases were under weight at delivery (1680–2290 Gm) with dry wrinkled and cracked skin. The placenta showed extensive infarction. The discrepancy between length of gestation and development of the children was tentatively suggested as being caused by impairment of placental function. The mean value for the peripheral vascular resistance for these 5 subjects was 39 PRU The corresponding mean value for normal pregnancy at this period was 10 PRU (Spetz, 1964)

The findings above seem to indicate a correlation between the degree of relative constriction of the resistance vessels in the forearm and the functional state of the placenta. A more detailed investigation is planned concerning the relation between the peripheral vascular resistance to blood flow and the function of placenta as estimated by measuring the excretion of oestriol and pregnanediol in the urine

In 1950 Burt studied forearm blood flow in 7 patients with pre-eclampsia These determinations were performed between the 33th and the 38th week of gestation. The mean value for resting forearm blood flow was found to be 4.47 ml. In 8 normal pregnant women studied between the 20th and the 41st week of pregnancy the corresponding mean value was 3.51 ml. On the basis of these values the investigator draws the conclusion that there must be an increase in skeletal muscle blood flow in pre-eclampsia However in view of the results presented here the blood flow values during normal pregnancy seem to be much higher than those indicated by Burt (1950)

In 1950 Burt also studied the forearm blood flow during

reflex vasodilatation placing the feet of the subject in a water bath at a constant temperature of 43 5 C. During this vasodilatation the blood flow in the pre-eclamptic group rose to higher values than for normal pregnancy These findings could not be confirmed in the present study

Some important factors should be mentioned which may influence the capillary filtration For a detailed discussion of these factors see Spetz (1965) The hydrostatic pressure in the capillaries to a great extent depends on the pre- to post-capillary resistance ratio In normal pregnant women this ratio was assumed to be 4 1 Although there is a dilatation of the resistance vessels during pregnancy there is reason to believe that the ratio remains unchanged (Spetz 1965) A decrease in the pre- to post-capillary resistance ratio would lead to a raised capillary pressure and, consequently to an increase of net outward filtration if no counteracting factors are involved. An accumulation of fluid in the extravascular space would tend to raise the tissue hydrostatic pressure In the present series there were however no signs of any marked increase in the tissue pressure. This was judged by the fact that no visible oedema was present in the forearm. Furthermore the filtration proceeded at a constant rate for 5-10 minutes when the capillary hydrostatic pressure was raised. If this pressure had been high, the fluid accumulation during the raised capillary pressure would lead to a rapid and considerably elevated tissue pressure and the rate of filtration would then tend to decline gradually The colloid osmotic pressure of the plasma proteins and the blood viscosity show slight variations in pre-eclampsia as compared with normal pregnancy However these variations seem to be of little quantitative importance (Lagercrantz 1945 Mack, 1960 DeAlvarez and Afonso 1964) Venous pressure in pre-eclampsia is within normal limits in the forearm (Thomson Reid, and Cohen 1939 McLennan 1943 b)

From the discussion above it seems logical to assume that the pre- to post-capillary resistance ratio in pre-eclampsia remains at about 4 1 In the present study the venous outflow pressure was increased by 20 mm Hg in measuring the net capillary filtration rate With this pre- to post-capillary resistance ratio only 16 mm

Hg can be calculated to be transmitted to the capillary level. However even if a small decrease in the pre- to post-capillary resistance ratio occurred this would not have changed the results presented.

Szontagh (1950) Abel (1960) and Friedberg and Lutz (1963) have given evidence of an increased capillary permeability in pre-eclampsia. If this were true, and provided that almost the same number of capillaries were open to flow the result would be a considerable increase in CFC as flow of liquid through pores is directly proportional to the fourth power of the diameter of the pore (Poiseuille's law) Such a considerable increase was not observed in this study and the results therefore do not favour the assumption that there is any change in capillary permeability This is in good accordance with the results given by Switzer Hester Milam, and Ownby (1950) They examined the protein content of the oedema fluid in pre-eclampsia and found low protein concentrations indicating that capillary permeability was not increased, at least not to proteins.

Capillary filtration rate in pre-eclampsia was studied by McLennan (1943 a, 1946) by means of pressure plethysmography Re-calculating his data with the assumption that 80 per cent of a given increase in venous outflow pressure is transmitted backwards to the capillaries, a mean value of CFC for 17 subjects in his study would be 0 0039 ml/min. × 100 ml × mm Hg. This value is somewhat lower than the mean value for normal pregnant women. McLennan (1943 a 1946) believed that the decrease in filtration rate compared with normal pregnancy was due to an elevated tissue pressure caused by oedema. The present study does not support this assumption

SUMMARY

1 In 38 cases of pre-eclampsia resting forearm blood flow and maximal blood flow capacity were examined by means of venous occlusion plethysmography Capillary filtration rate in the forearm was studied on the same occasions by a volumetric method.

2 In pre-eclampsia there was a tendency to increasing values of forearm blood flow towards the end of pregnancy The values

were however always lower than the corresponding values for normal pregnancy The differences were statistically significant

3 In pre-eclampsia there was in the forearm a statistically significant increase in the vascular resistance to blood flow in comparison with normal pregnancy A correlation between relatively high values for vascular resistance and disturbances of the growth of the foetus was assumed to exist.

4 No change in the "maximal blood flow capacity in pre-eclampsia in relation to normal pregnancy could be proved. The resistance vessels in the two series could dilatate to the same extent.

5. The mean value of capillary filtration coefficient in 22 cases of pre-eclampsia was 0 0048 ml/min × 100 ml × mm Hg There is no statistical significant difference between this value and a corresponding mean value for normal pregnancy 0 0052 ml/min × 100 ml × mm Hg Different factors which can influence the capillary filtration rate in pre-eclampsia are discussed.

Acknowledgements

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THE EFFECT OF ŒSTROGEN TREATMENT ON MYOMETRIAL ACTIVITY IN LATE PREGNANCY

BY

PENTTI A. JÄRVINEN TAPANI LUUKKAINEN AND LEILA VÄISTÖ

The role of steroid hormones in the onset of labour has been well reviewed recently (Bengtsson Diczfalusy Fuchs, 1962) Current knowledge suggests that the onset of labour is not preceded by a clear change endogenous steroid production and exogenous systemic steroids fail to postpone the onset of labour Œstrogens have been widely used for the induction of labour and their effectiveness has been suggested by many obstetricians (Robinson *et al* 1935 Jeffcoate 1950 Kalkschmied, 1958 and 1960 Tappfer 1959 Vara *et al.* 1961 Pinto *et al.*, 1964) Trials with statistically significant results, of the effects of intramuscular œstrogen on the progress of induced labour have been reported previously from this hospital (Järvinen and Luukkainen 1962 Järvinen and Huhmar 1963) The trials reported by Klopper and Dennis 1962 did not show a positive correlation between their mode of treatment and the induction-delivery interval but the method of statistical analysis was different from ours.

While treatment with œstradiol given intramuscularly was shown to facilitate the induction of labour it was thought desirable to assess whether this effect was achieved by the stimulation of the myometrial activity

Material and Methods

Forty-nine women at 35–43 weeks gestation and aged 18–38 years were selected from patients admitted to hospital for induc-

Table I. *Uterine activity recorded with external tocograph before and after oestrogen treatment. Uterine activity units = mean of contractions in g/cm² multiplied by number of contractions. Area units = area of contractions in mm² from the tocograph tracing during 40 minutes*

Case	Gestation (Weeks)	Number of Contractions during 40 Minutes		"Uterine Activity Units" during 40 Minutes		Area Units in mm² during 40 Minutes	
		Before	After	Before	After	Before	After
1	42	3	3	51	61	1020	1320
	39	3	3	55	58	999	1368
3	39	9	6	45	185	405	2976
4	43	4	5	64	120	1216	3060
5	38		8		56	935	952
6	42	4	4	73	39	1746	634
7	40	7	16	29	204	303	4368
8	35	7	9	35	63	675	417
9	4	6		4	205	576	2448
0	43	3	9	157	175	4	620
	40		3		85	250	560
12	37			40	48	60	144
13	4	5		55	96	532	720
14	40	6	4	67	78	952	1660
5	39		2	1	29	65	660
16	39	3	3	25	25	360	500
17	38		9	6	66	78	2454
8	39		4	0	70		260
9	42		3	16	73	336	050
20	40			0	54		1430
	38	4	5	80	120	240	1740
22	4	3	3	55	31	998	2706
3	39		4	25	59	725	432
24	4	3	4	23	70	46	368
25	43	3	2	38	80	624	4600
26	34				8		60
27	4		4	16	88	44	3 24
Mean	39 6	4	5 6	45 6	83 3	679	1654

tion of labour Patients with clinically evident uterine contractions with ruptured membranes or with a dilated cervix (as judged by rectal examination) were excluded. The selected patients were observed for one day The next morning at 9 a m. uterine activity was recorded for one hour with an external guard ring tocograph (Stanley Cox, Ltd England) designed by Smyth (1957)

The first and last ten minutes of the recording were discarded, and in the remaining recording period of 40 minutes the number of contractions was observed and their intensity in g/cm² and area in mm² were measured The mean intensity of contractions was multiplied by the number of contractions to give uterine activity units" during 40 minutes This calculation resembles the measurement of uterine activity introduced by Caldeyro Barcia *et al.* (1957) called "Montevideo units" This name is not used in the present report because an external recording of uterine pressure in g/cm² in a 40 min period is used instead Caldeyro-Barcia's technique of recording uterine pressure in mm Hg intra-amniotically during 10 minutes The area units used in this report are the total area in mm² from the tocograph tracing of uterine contractions in 40 minutes resembling the active pressure area cm²/30 minutes" introduced by Csapo *et al* (1963) with intra-amniotic recording

After these basal recordings 37 patients were each given five intramuscular injections of 5 mg of oestradiol benzoate at intervals of two hours (total 25 mg) and 12 patients were each given five intramuscular injections of 1 ml of 5 per cent dextrose solution at intervals of two hours The one-hour recording was repeated on the following morning at 9 a m. and the myometrial activity was measured during 40 minutes as before Immediately after the recording amniotomy was performed Ten patients with oestrogen and two patients from the control series delivered spontaneously before the second recording and these were excluded from the study Fourteen of the remaining 27 patients treated with oestrogen and 3 of the 10 control patients were primigravidae All patients in the study gave birth to living children weighing 2570–4630 g without any medical or surgical intervention except episiotomy

Results

The data from recordings of uterine activity before treatment with oestrogens and on the following morning are given in Table I The values for the control group are detailed in Table II

The mean of the uterine activity units in the basal recording

was 45.6 in the group treated with oestrogen and 46.3 in the control group. The area units were 679 and 791 respectively.

The effect of treatment with oestrogens can be evaluated by counting the number of cases in each parameter studied in which the value increased and in which it was unchanged after the treatment, and by comparing them with similar observations in the control group.

In the oestrogen treated group the number of contractions increased in 17 cases and remained unchanged or decreased in 10 cases. In the control group there was an increased number of contractions in 2 out of 10 cases. The mean values in the two groups show a similar trend. The uterine activity units were increased in 19 out of 27 cases in the oestrogen treated group and in 4 out of 10 cases in the control group. The area units, were increased in 23 cases and remained unchanged or decreased in 4 cases in the oestrogen treated group. In the control group there was an increase in 4 cases and no change or a decrease in 6 cases. The mean values of the area units in the oestrogen treated group also show an increase after treatment. It seems that oestrogen treatment increased the uterine activity more than dextrose treatment, as judged by every parameter studied.

Statistical analysis of the data in these tables was carried out as follows. The absolute difference of the area units before and after oestrogen or dextrose treatment was calculated in every case. These differences were rearranged into columns in the order of superiority in both groups. An ordinal number was given to them so that the first had the greatest negative value and the last the greatest positive value. These were called Rank I. Then in both groups the area units before treatment were divided in every case by the area units after treatment. They were rearranged in the order of superiority and given an ordinal number in similarity to the differences and the column of the ordinal numbers was called Rank II. The numbers of Rank I and II were added in every pair and after this calculation the numbers were rearranged again in the order of superiority (Rank III). These values were statistically analyzed, and it was found that the values of the control group were on a significantly lower level ($P < 0.01$).

The values of the uterine activity units were analyzed simi-

Table II *Uterine activity recorded with external tocograph before and after dextrose treatment Uterine activity units = mean of contractions in glcm² multiplied by number of contractions. Area units = area of contractions in mm² from the tocograph tracing during 40 minutes*

Case	Gestation (Weeks)	Number of Contractions during 40 Minutes		"Uterine Activity Units" during 40 Minutes		Area Units in mm² during 40 Minutes	
		Before	After	Before	After	Before	After
28	42	9	2	125	43	1554	1012
29	41	4	1	22	8	276	480
30	39	3	2	29	56	630	1070
31	39	3	3	80	95	1499	1438
32	38	0	0	0	0	0	0
33	42	1	3	20	56	350	639
34	40	6	3	84	39	1890	999
35	38	3	10	76	279	1376	3640
36	36	0	0	0	0	0	0
37	38	1	0	27	0	390	0
Mean	39.3	3.2	2.4	46.3	57.6	791	932

larly and the result was the same There was a statistically significant increase in these values as compared with the control group but on a lower level of significance ($P < 0.02$)

The median of the induction-delivery interval for oestrogen treated patients was one hour shorter than that of controls

Discussion

In the investigation of a series of obstetric patients near term, it is difficult to achieve significant evidence concerning the effectiveness of a treatment which may facilitate the onset of labour because pregnancy is not a static condition The dynamic progressive nature of late pregnancy with great individual variation makes it understandable that the role of the individual components of the complex hormonal regulation of labour has not been clarified.

When the patients are treated with oestradiol benzoate and the results expressed as a progressive percentile it is observed that the induction-delivery interval is shorter in oestrogen treated patients than in control patients (Järvinen and Luukkainen

1962) This beneficial effect is not accompanied by increased sensitivity to oxytocin (Järvinen and Huhmar 1963) These facts do not demonstrate that œstrogens have any physiological role in normal labour nor which physiological mechanism may mediate this effect. The present work shows that the effect may be based on stimulation of myometrial activity This increase in uterine activity seems to be slight, as in physiological conditions before the onset of the labour but enough to precipitate labour in those patients in whom the other regulatory mechanisms favour the onset of labour It was observed, that 10 out of 37 patients commenced labour spontaneously during the night after treatment with œstrogens. A similar effect was observed also in previous work in our hospital (Vara *et al.*, 1961)

The basal biochemical mechanism through which this physiological phenomenon operates is obscure. However it could be anticipated that many enzymic reactions in the maternal organism and in the myometrium change their reaction rates after the treat ment. Therefore it seems unlikely that the activation was based on the direct immediate effect on the myometrium observed by Pinto *et al* (1964) with intravenous œstradiol infusion. Further it seems doubtful that any change occurred in two parameters, uterine volume or placental progesterone concentration whose ratio according to Csapo *et al* (1963) control the uterine activity Thus it seems that none of the existent theories alone could explain the onset of labour and much further work is called for to elucidate the many unknown problems in the complex regulation of the onset of labour

SUMMARY

The basal uterine activity was recorded by means of an external tocograph in 49 women of gestation periods ranging from 35–43 weeks. Œstrogen treatment with 25 mg of œstradiol benzoate intramuscularly was given to 37 women and dextrose solution was given to 12 women Ten out of 37 women in the œstrogen treated group and 2 out of 12 in the control group had a spontaneous delivery before the second recording on the following

morning The statistical analysis showed a significant stimulation of the myometrial activity in the oestrogen treated group as compared with the control series

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UTERINE MOTILITY AND RESISTANCE OF THE LOWER PARTS OF THE UTERUS AT THE ONSET OF LABOUR

BY

LENNART LINDGREN AND NILS FAGERLUND

Cervical dilatation during labour is controlled by three main factors: the strength of contractions, the frequency of contractions and the resistance of the lower parts of the uterus. The amniotic pressure and the frequency of contractions, i.e. the uterine activity is dependent on the response to oxytocin. At the same uterine activity the most rapid spontaneous cervical dilatation occurs with contractions at a frequency of 21–23 per hour (Lindgren and Smyth, 1961; Lindgren 1962). In uterine fibrillation and hypotonic inertia no progress in labour was observed when the average amniotic pressure was below 25 mm Hg (Caldeyro Barcia et al. 1955; Lindgren and Ingelman Sundberg, 1959; Lindgren, 1960). Increased cervical resistance as in spastic contraction of the lower uterine segment requires a higher amniotic pressure to effect dilatation than is the case with normal labour (Lindgren 1962). This resistance may be a persistent muscle cone described by Shaw and Nirula (1951) and by Wendell-Smith (1954). We know that the increased resistance of a rigid cervix delays labour. On the other hand premature labour may result from decreased resistance of the lower parts of the uterus as for example in association with isthmic insufficiency, a soft cervix or after high amputation of the cervix during surgical repair for prolapse.

In an earlier investigation (Lindgren 1961) it was pointed out that the frequency of contractions at the onset of labour

morning The statistical analysis showed a significant stimulation of the myometrial activity in the oestrogen treated group as compared with the control series.

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pressure was measured and the frequency of contractions was estimated for half an hour before cervical dilatation was evident by vaginal examination. Where no cervical dilatation could be determined, the amniotic pressure and frequency of contractions were estimated for half an hour during the administration of 15 mU per min. of syntocinon®

The material was divided into 4 groups normal labour with spontaneous cervical dilatation successful syntocinon induction unsuccessful syntocinon induction and spastic contractions of lower uterine segment.

The age of the mother the foetal birth weight and the occipitobregmatic circumference for the different groups are given in Table I. All patients were examined at term with foetus presenting by the head.

RESULTS

Normal labour with spontaneous cervical dilatation

The amniotic pressure was recorded in 43 patients of whom 35 were primigravidae The frequency of contractions and the uterine activity were calculated. The uterine activity varies in different patients at the onset of cervical dilatation (Fig. 1) In this figure the uterine activity of patients with spontaneous dilatation has been compared with the uterine activity of patients scheduled for induction of labour but before the administration of oxytocin. From the diagram it is evident that in some patients the uterine activity was only 30–40 M.U during dilatation but in other cases no dilatation was observed in spite of an uterine activity of 100–110 M.U In Figure 2 the amniotic pressure has been correlated with the corresponding frequency of contractions in the same patients. No cervical dilatation was observed if the average amniotic pressure was below 24 mm Hg In all cases with amniotic pressure over 30 mm Hg the cervix started to dilate The frequency of contractions for dilatation to occur must however be more than 11 per hour Single strong contractions of Braxton Hicks contractions do not dilate the cervix All the patients with spontaneous dilatation showed regular contractions.

Table I. *The Material*

	Number of Patients	Age of the Mothers (yr)	Birth Weight (g)	Occipito-bregmatic Circumference (cm)
Spontaneous dilatation with normal labour				
Primigravidæ	35	25.2 ± 0 8	3450 ± 70	33.4 ± 0.2
Multigravidæ	8	27 9 ± 2.1	3370 ± 100	33 9 ± 0.2
Induced labours (Intravenous syntocinon®)				
Primigravidæ	12	28.3 ± 2 7	3420 ± 145	33 2 ± 0.3
Multigravidæ	13	32.5 ± 1 7	3870 ± 80	34.5 ± 0.2
Intravenous syntocinon® (No cervical dilatation) Primigravidæ	8	28 6 ± 2.6	3540 ± 155	34.0 ± 0.3
Multigravidæ	30	33 9 ± 1 3	3640 ± 60	34.2 ± 0.2
Spastic contractions of the lower uterine segment Primigravidæ	5	26.2 ± 2.5	3470 ± 250	33.3 ± 0.8

varies within wide limits Low initial frequency is followed by a slow first stage and high frequency by more rapid cervical dilatation An average frequency *i e* 16–24 contractions per hour at the onset of labour was seen in association with short first stage. Also uterine activity varies within wide limits at the onset of labour 80–120 M. U (Montevideo units) according to Caldeyro Barcia *et al* (1958)

The intention of the present investigation is to obtain information about the uterine motility and the resistance of the lower uterine segment at the spontaneous onset of labour and in induced labour

METHOD AND MATERIAL

The method of intrauterine tocography elaborated by Ingelman Sundberg and Lindgren (1955) was used in spontaneous labour and in labour induced by syntocinon® the contractions were recorded by Smyth s guard-ring tocograph (1957) For induction of labour intravenous infusion of syntocinon® was used in increasing doses of 4 8 6 7 2 9, 12 and 15 mU per min Each dose rate was continued for half an hour The amniotic

pressure was measured and the frequency of contractions was estimated for half an hour before cervical dilatation was evident by vaginal examination Where no cervical dilatation could be determined, the amniotic pressure and frequency of contractions were estimated for half an hour during the administration of 15 mU per min. of syntocinon®

The material was divided into 4 groups normal labour with spontaneous cervical dilatation successful syntocinon induction unsuccessful syntocinon induction and spastic contractions of lower uterine segment.

The age of the mother the foetal birth weight and the occipito-bregmatic circumference for the different groups are given in Table I. All patients were examined at term with foetus presenting by the head.

RESULTS

Normal labour with spontaneous cervical dilatation

The amniotic pressure was recorded in 43 patients, of whom 35 were primigravidae. The frequency of contractions and the uterine activity were calculated. The uterine activity varies in different patients at the onset of cervical dilatation (Fig. 1) In this figure the uterine activity of patients with spontaneous dilatation has been compared with the uterine activity of patients, scheduled for induction of labour but before the administration of oxytocin. From the diagram it is evident that in some patients the uterine activity was only 30–40 M.U during dilatation but in other cases no dilatation was observed in spite of an uterine activity of 100–110 M U In Figure 2 the amniotic pressure has been correlated with the corresponding frequency of contractions in the same patients. No cervical dilatation was observed if the average amniotic pressure was below 24 mm Hg In all cases with amniotic pressure over 30 mm Hg the cervix started to dilate The frequency of contractions for dilatation to occur must, however be more than 11 per hour Single strong contractions of Braxton Hicks contractions do not dilate the cervix. All the patients with spontaneous dilatation showed regula contractions.

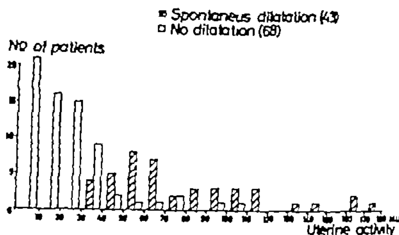


Fig. 1 Uterine activity at the onset of cervical dilatation.

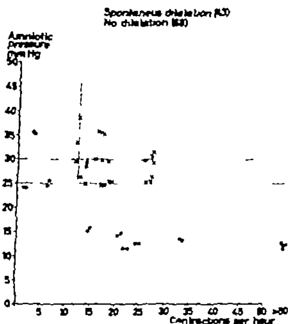


Fig 2. The average amniotic pressure and the corresponding frequency of contractions at the onset of cervical dilatation.

The head to cervix pressure at the largest circumference of the foetal head (LS) and 3 cm below this level (LS_1) was recorded in 10 patients of whom 5 were primigravidæ (Fig 3) The head to cervix pressure was correlated with the correspond

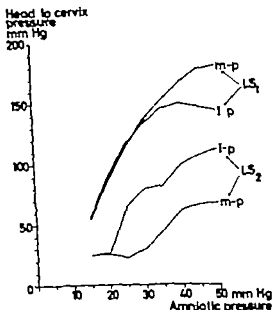


Fig 3 The average head to cervix pressure and the corresponding amniotic pressure at the onset of cervical dilatation.
LS —The largest circumference of fetal head.
LS —3 cm below LS -level.
I-p —Primigravidae
M-p—Multigravidae

ing amniotic pressure At the largest circumference of the head no difference could be demonstrated between multigravidae and primigravidae at an average amniotic pressure below 30 mm Hg, but at higher amniotic pressures the multigravidae showed higher head-to-cervix pressures than primigravidae. In these groups of patients the correlation curve deviates asymtotically at higher amniotic pressures. Also at the level 3 cm below the largest circumference of the head no difference in the head to cervix pressure could be demonstrated between primigravidae and multigravidae at low amniotic pressures. But at higher amniotic pressures the primigravidae showed higher readings in comparison with the multigravidae i e the opposite of the findings at the largest circumference of the head. No significant difference in

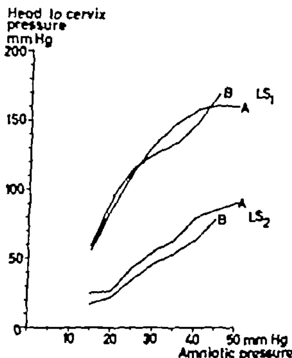


Fig. 4. The average head to cervix pressure and the corresponding amniotic pressure in primigravidæ and multigravidæ.

LS —The largest circumference of foetal head.

LS —3 cm below LS_1-level.

A —At onset of cervical dilatation (half an hour)

B —Recording during the half an hour before A.

the average amniotic pressure between primigravidæ and multigravidæ could be demonstrated The amniotic pressure and the head to cervix pressure were also measured during the half an hour period before the period when cervical dilatation commenced The pressures were compared with the corresponding average pressures when the cervix was dilated (Fig 4) At low amniotic pressures no difference could be demonstrated between the head to cervix pressures at the largest circumference of the head Before cervical dilatation at higher amniotic pressures the correlation curve did not show such an asymtotic deviation when the cervix started to dilate Also at the lower level the correlation curve showed no asymtotic deviation at higher amniotic pressure and the correlation curve between the head to cervix

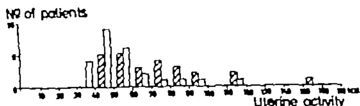


Fig. 5. The uterine activity at the onset of cervical dilatation by intravenous syntocinon®
At no dilatation the recording was made during the half an hour preceding the onset of cervical dilatation.

pressure and the amniotic pressure was approximately a straight line

All the patients with spontaneous cervical dilatation had a ripe cervix i e softening and effacement.

Successful and unsuccessful induction of labour

Intravenous syntocinon®-infusions were given to 63 patients in increasing doses up to 15 mU per minute as described previously In 25 patients of whom 12 were primigravidae the cervix dilated and the patients were delivered within 24 hours. The indications were post-maturity toxaemia or Rh-immunization.

The amniotic pressure was measured and the frequency of contractions was estimated for the half hour before cervical dilatation started. The uterine activity was calculated in each patient. These values were compared with the corresponding values obtained in the preceding half hour period. The uterine activity varied within wide limits, as was seen in association with spontaneous dilatation (Fig 5) In Figure 6 the average amniotic pressure has been correlated with the frequency of contractions

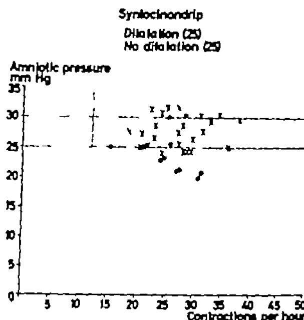


Fig. 6 The average amniotic pressure and the corresponding frequency of contractions at the onset of cervical dilatation by intravenous syntocinon® At no dilatation the recording was made during the half an hour preceding the onset of cervical dilatation.

From the diagram it is evident that no cervical dilatation was observed when the average amniotic pressure was below 24 mm Hg and that the cervix dilated in all cases when the average amniotic pressure was more than 30 mm Hg, *i e* the same observation as were made in cases of spontaneous dilatation in normal labour

Uterine activity in the group of patients in whom the cervix failed to dilate in spite of intravenous syntocinon® in doses up to 15 mU per min is shown in Figure 7 The uterine activity of successfully induced labours also is plotted in this figure. The average amniotic pressure was correlated with the frequency of contractions and is compared with the group of successfully in duced labours in Figure 8 From the figure it is seen that the cervix failed to dilate in spite of an average amniotic pressure over 30 mm Hg and a frequency of more than 11 contractions per hour

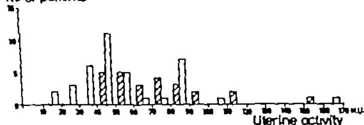


Fig. 7 The uterine activity at the onset of cervical dilatation by intravenous syntocinon®

At no dilatation 5 mU per min. of syntocinon® was given.

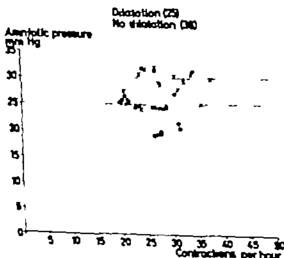


Fig. 8 The average amniotic pressure and the corresponding frequency of contractions at the onset of cervical dilatation by intravenous syntocinon®

At no dilatation 15 mU per min. of syntocinon® was given.

Table II. *The Uterine Response to Oxytocin (The figures in parentheses show the number of patients with successful induction.)*

Low frequency ⋜ 11 contractions per hour
Low amniotic pressure ⋜ 24 mm Hg
Regular contractions > 11 contractions per hour and > 24 mm Hg of average amniotic pressure.

	Before Syntocinon Infusion	After Syntocinon® Infusion			
		Low Frequency Amniotic Pressure > 4 mm Hg	Irregular Contractions	Low Amniotic Pressure Contractions > per H	Regular Contractions
Primigravidæ					
Low frequency	2	–	–	–	2 (2)
Irregular contractions	9	–	–	3 (0)	6 (5)
Low amniotic pressure	9	–	3 (0)	1 (0)	5 (5)
Multigravidæ					
Low frequency	24	1 (0)	–	4 (0)	19 (6)
Irregular contractions	8	–	1 (0)	1 (0)	6 (5)
Low amniotic pressure	11	–	–	3 (0)	8 (2)
All patients					
Low frequency	26	1 (0)	–	4 (0)	21 (8)
Irregular contractions	17	–	1 (0)	4 (0)	12 (10)
Low amniotic pressure	20	–	3 (0)	4 (0)	13 (7)

Table II shows the type of motility before and after intravenous syntocinon® in the group of induced labours and in the groups where no cervical dilatation could be demonstrated. Incordinate contractions are more common in primigravidæ and Braxton Hicks contractions in multigravidæ in all groups. In the group with no cervical dilatation in spite of syntocinon® low frequency was seldom observed but low amniotic pressure was not so uncommon. In only one case irregular contractions failed to become regular

In Table III the "ripeness of the cervix in the different groups is presented It is notable that failure of dilatation with intravenous

Table III. *The Distribution of the Patients with Regard to the "Ripeness" of the Cervix*

	Number of Patients	Effaced and Soft Cervix	Uneffaced and Soft Cervix	Effaced and Rigid Cervix	Uneffaced and Rigid Cervix
Spontaneous dilatation Primigravidae	35	35		0	0
Multigravidae	8	8	0	0	0
Induced labour					
Primigravidae		9	2		
Multigravidae	3	5	6		
No dilatation					
Primigravidae	8			1	7
Multigravidae	30		0	7	13

Table IV. *The "Ripeness" of the Cervix and the Amniotic Pressure during Induction of Labour by Intravenous Syntocinon® (The figures in parenthesis show the number of patients with successful induction.)*

		Number of Patients	Effaced and Soft Cervix	Uneffaced and Soft Cervix	Effaced and Rigid Cervix	Uneffaced and Rigid Cervix
Primigravidae	< 24 mm Hg	6		–	( )	5 ( )
	24–30	8	6 (6)	( )	( )	1 ( )
	> 30	5	3 (3)	( )	0	1 ( )
Multigravidae	< 24	9	–		2 ( )	7 (0)
	24–30	26	6 (6)	( )	8 (4)	0 (0)
	> 30	8		(1)	2 ( )	5 (0)
All patients	< 24	5		–	3 (0)	12 (0)
	24–30	35	(2)	( )	(5)	2 ( )
	> 30	13	3 (3)	( )	( )	8 ( )

syntocinon was commonly associated with a rigid and uneffaced cervix whereas there was no failure of dilatation when the cervix was soft.

In Table IV the distribution of the cases is given according to the "ripeness" of the cervix and the variations in the amniotic pressure with administration of oxytocin. In every patient with a soft cervix the average amniotic pressure was more than 24 mm Hg. If the cervix was rigid but effaced the average amniotic pressure was more than 24 mm Hg in 80 per cent of cases. If

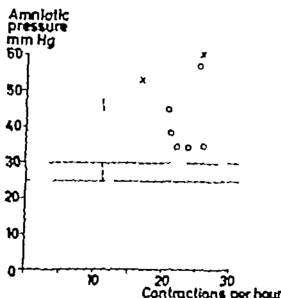


Fig. 9. The average amniotic pressure and the corresponding frequency of contractions at the onset of cervical dilatation in patients with spasm of lower uterine segment.

X Dilatation (5 primigravidae)

O No dilatation. Recordings from the same patients during the half hour preceding cervical dilatation.

the cervix was both uneffaced and rigid the average amniotic pressure was more than 24 mm Hg in only 61 per cent of cases. The difference between the response to oxytocin with ripe and "unripe" cervices was statistically significant ($P < 0.001$) In patients with no cervical dilatation and an average amniotic pressure of more than 30 mm Hg the cervix was both rigid and uneffaced in all except one case

Spastic contractions of lower uterine segment

The amniotic pressure and the head to cervix pressure was measured in 5 patients with spastic contractions of the lower uterine segment. The age of the mother the birth weight and the occipito-bregmatic circumference are given in Table I

In Fig 9 the average amniotic pressure and the corresponding frequency of contractions is plotted. The pressures were measured during the half an hour before evidence of cervical dilatation was found and compared with the average amniotic pressure

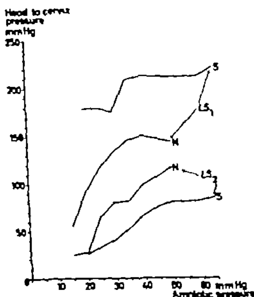


Fig. 10 The average head to cervix pressure and the corresponding amniotic pressure at the onset of cervical dilatation in primigravidae.

LS —The largest circumference of foetal head

LS —3 cm below LS -level

N —Normal contractions

S —Spastic contractions of lower uterine segment.

during the half an hour preceding this. Average amniotic pressures were invariably over 30 mm Hg, notably higher than the pressures found in normal labour The average pressure between the largest circumference of foetal head and lower uterine segment was higher than in normal labour (Fig. 10) The asymtotic deviation of the correlation curve at higher amniotic pressures was lacking in the presence of spasm. The high head to cervix pressure in association with low amniotic pressure is notable At lower levels (LS_2) the head to cervix pressures were lower than the corresponding pressures in normal labour

DISCUSSION AND CONCLUSIONS

The investigation shows that an average amniotic pressure of 24 30 mm Hg and a coexisting frequency of more than 11–12

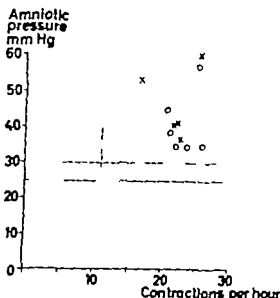


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In Fig 9 the average amniotic pressure and the corresponding frequency of contractions is plotted. The pressures were measured during the half an hour before evidence of cervical dilatation was found and compared with the average amniotic pressure

SUMMARY

The average amniotic pressure was measured by means of intrauterine tocography and the frequency of contractions was estimated during the half hour preceding cervical dilatation as judged by vaginal examination in forty-eight patients. There were 13 primigravidæ, of whom 8 had normal spontaneous dilatation and 5 had spontaneous dilatation but showed spasm of the lower uterine segment. In 5 primigravidæ and 5 multigravidæ with regular contractions and 5 primigravidæ with spasm of the lower uterine segment pressures were measured also between the lower part of the uterus and the foetal head. Sixty-three patients of whom 20 were primigravidæ were admitted for induction of labour In 12 primigravidæ and 13 multigravidæ the induction was successful. Their contractions were studied using the guard-ring tocograph as described by Smyth.

1 The cervix starts to dilate both spontaneously and during induction of labour when the average amniotic pressure reaches 24 30 mm Hg and there is a coexisting frequency of at least 11–12 contractions per hour No significant difference between primigravidæ and multigravidæ could be demonstrated. The cervix must be ripe i.e soft and effaced.

2 The head to cervix pressure increases proportionally at low amniotic pressures at the onset of cervical dilatation At higher amniotic pressures the correlation curve deviates asymtotically in the normal first stage. This is an expression of the fact that the uterine tissue is overdistended or partly ruptured, the prerequisite condition for cervical dilatation. At higher amniotic pressures the resistance of the uterine wall at the largest circumference of the foetal head is greater in multigravidæ than in primigravidæ but below this level the resistance is less in multigravidæ i.e the force of cervical dilatation is more effective at multigravidæ

3 In patients with spasm of the lower uterine segment the resistance to cervical dilatation is higher than in normal labour A higher amniotic pressure is necessary for cervical dilatation.

contractions per hour is necessary for cervical dilatation in normal patients who have a ripe cervix at the onset of labour Observations were made during spontaneous dilatation and during induction of labour by intravenous syntocinon© infusion The investigation confirms the observation of Caldeyro Barcia et al. that the uterine activity varies within wide limits at the onset of labour There is no significant difference in the amniotic pressures at the onset of labour between primigravidæ and multigravidæ The correlation between the head to cervix pressure at the *largest circumference* of the head and the *amniotic pressure* is proportional at low amniotic pressures but the correlation curve deviates asymtotically at higher amniotic pressures. This illustrates the fact that at higher amniotic pressures the lower part of the uterus is overdistended or partly ruptured, the prerequisite condition for cervical dilatation At high amniotic pressures the pressure at the largest circumference of the foetal head is higher in multigravidæ than in primigravidæ At lower amniotic pressures the corresponding cephalo-cervical pressure is lower in multigravidæ than in primigravidæ This fact explains the higher resistance of the uterine tissue at the largest circumference of foetal head in spite of the same amniotic pressure *i.e.* the force of dilatation in multigravidæ is more concentrated at the largest circumference of foetal head.

No asymtotic deviation of the correlation curve of the head to cervix pressure and amniotic pressure could be demonstrated before the onset of labour Uterine motility in response to oxytocin seems to be related to the ripeness of the cervix In some cases however response to oxytocin is good but if the cervix is unripe the increased resistance to cervical dilatation results in failure of induction The corresponding condition exists in spastic contractions of the lower uterine segment where also higher amniotic pressure is necessary to overcome the increased resistance to cervical dilatation

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CONSERVATIVE TREATMENT OF THREATENED PREMATURE LABOUR

BY

K. SOIVA AND O. CASTRÉN

In spite of improved paediatric treatment the neonatal mortality rate amongst premature infants is still unduly high. Even value of prolonged successful and expensive treatment is reduced, particularly as far as the lower weight classes are concerned, by the abundance of late neurological disorders. In Eastman's study (1962) there were 20 times as many premature infants weighing less than 1500 g, in the group of children with cerebral palsy as in the control group. Continuation of intra-uterine development even for two weeks greatly improves the neonatal prognosis in many cases. Among 100 premature liveborn infants weighing under 1500 g, 46 died within the first 24 hours and at the end of the 4th week only 34 were still alive, whereas in a group weighing 1500–2000 g the mortality during the first twenty four hours was reduced by three quarters. At the end of the first month twice as many infants in the 1500–2000 g were alive as compared with the < 1500 g weight group (Donald, 1964). It is obvious that the results could be effectively improved only by preventing threatening late abortion or premature delivery. Surgical treatment by Shirodkar's operation or its modifications makes it possible in some relatively rare cases of incompetence of the internal os of the cervix to continue the pregnancy to calculated term. In the majority of cases in which the aetiology remains obscure, such a measure cannot be adopted. Instead one must attempt to prevent premature delivery by conservative

4 At the onset of labour at term incoordinate contractions are more common in primigravidæ and Braxton Hicks contractions are more frequent in multigravidæ

5 The uterine response to oxytocin seems to be well correlated to the "ripeness" of the cervix. Sometimes however the cervix is unripe and the uterine contraction response to oxytocin is good although in these cases the cervix does not dilate

6 The "unripe" cervix and contractions of the lower uterine segment impair cervical dilatation A third factor which prevents labour is an abnormal uterine response to oxytocin in the form of small frequent contractions

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Table I. *Age Parity Duration of Pregnancy (Weeks) and Indication for Admission to Hospital*

		A	B	C
Age	20	6	8	3
	20–29	21	40	27
	30–39	8	13	
	40	–	3	5
Parity	I	18	27	20
	II	7	18	11
	III	7	10	9
	IV	1	9	7
Pregnancy-weeks				
	25–26	5	5	7
	27–28	6	8	6
	29–30	8	8	10
	31–32	5	14	13
	33–34	9	29	1
Signs of premature labour				
	contractions	22	46	26
	haemorrhage	5	17	1
	rupture of membranes	19	37	18
Total		33	64	47

in addition as well as progesterone in some cases, and in A nearly half of the patients were given Allylestrenol (= 17α-allyl-17β-hydroxy-estr-4-ene = Gestanon®) (5 mg 4–6 times daily). In B this medication was administered to only 10 per cent. In C the therapy consisted, in addition to bed rest, of allylestrenol given to all (5 mg 6–8 times daily). In B and C experiments were also made in some cases with isoxsuprin administered by intravenous drop infusion (Hendricks *et al.* 1961) or by intramuscular injection. The distribution of the patients according to parity pains, escape of the amniotic fluid, haemorrhage and duration of pregnancy is shown in Table I. In assessing the cases consideration should be given to the circumstance that unit B had a preponderance of more complicated cases (e.g. Rh-immunisation) whereas in A most patients were otherwise normal. Series B contained relatively more 33–34 weeks cases than the others.

methods in spite of the onset of pains, haemorrhage and/or rupture of the membranes. The interest in these attempts is naturally considerable (e.g. Bishop and Woutersz, 1961, Borglin and Eliasson 1962, Brenner and Hendricks 1962, Bueman and Lange 1962, Fuchs and Stakemann 1959, Willemsen, 1960, Øvlisen and Iversen, 1962) although the value of such therapy and particularly that of progesterone (Fuchs and Stakemann 1959, Bengtsson and Fuchs 1962) and of some synthetic derivatives of it (Brenner and Hendricks, 1962) is sometimes questionable. There is a tendency to accept as evidence only double-blind controlled studies which, being difficult to carry out in practice, are generally not so extensive that the final result remains uninfluenced by such accidental factors as, for example, foetal malformations which may unavoidably lead to interruption of pregnancy. Nevertheless, for a practical working obstetrician it is of interest to see what methods different institutions adopt in attempts to postpone threatening abortion and to obtain a living child who remains alive at least over the neonatal period.

Material

The series was collected from three different maternity hospitals (The Maternity Hospital of the city of Turku = A, the University Central Hospital, Turku = B and the Central Hospital of Tampere = C). From A and B for the period 1961, 1962 and 1963 and from C for 1962 (the year the hospital was opened) and 1963. All those patients were included whose pregnancy had continued with certainty for a minimum of 25 weeks or for maximum of 34 weeks (according to Naegele's rule) and who had to be admitted because of threatened late abortion or premature labour (unmistakable pains, haemorrhage and/or rupture of the membranes). Twin pregnancies (= 20 patients) and triplet pregnancies (= 3 patients) and patients who were delivered within 24 hours of admission were excluded from the series. In the remaining cases attempts were made to continue the pregnancy by conservative treatment. The patients were kept at rest in bed, in A and B sedatives and morphine (or its derivatives) were used

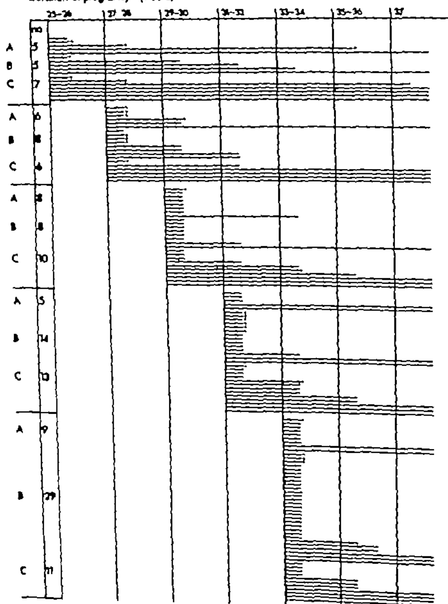


Fig. Duration of pregnancy on admission to hospital and continuation of pregnancy in series A, B and C. (+ = perinatal death)

Table II. *Stage of Pregnancy, Latent Period Exceeding Two Weeks and Live Births*

Stage of pregnancy	Series	No. of Cases	Latent Period Exceeding Weeks	Live Births
	A	5	2	2
25–26	B	5	4	3
	C	7	5	4
	A	6	1	2
27–28	B	8	2	3
	C	6	5	4
	A	8	1	7
29–30	B	8	1	4
	C	10	6	9
	A	5	2	5
31–32	B	14	2	7
	C	13	5	11
	A	9	2	6
33–34	B	29	3	26
	C	11	4	11

Results

If delivery occurred within 2 weeks after the admission it was considered that therapy had failed. If the pregnancy continued for at least two weeks after admission, within which time the weight of the foetus probably increased by 400 g or more (Buemann and Lange 1962) with a consequent improvement in foetal prognosis, the therapy was considered to be successful (Table II and Figure 1). Such successful therapeutical results were obtained in series A in about one fourth of the cases (8/33), in series B in one fifth (12/64) and in series C more than half (25/47). In all the series delivery was postponed irrespectively of the therapy employed in 30 per cent.

A living child who remained alive over the neonatal period was obtained in series A (22/33) and B (43/64) in about two thirds of the cases and in series C (39/47) in four fifths. If we disregard the last two week period (33rd–34th week), in which the foetal prognosis even without postponement of delivery is

32nd week, perinatal mortality was 22 per cent in series C compared with 51 per cent in series A and B. Although these results were affected by numerous factors (Solva and Castrén) there is obviously justification for continuing experiments with gestagens. Since it would appear that no great success in preventing the births of premature infants has been achieved by improvements in antenatal care (Pitkänen 1962) – in Finland the incidence of prematurity has remained at about 5 per cent – it is advisable to continue the study of the possible therapeutic measures (Backman and Unnérus 1963) for preventing threatening premature delivery at maternity hospitals.

SUMMARY

Among 144 patients with a duration of pregnancy of 25 to 34 weeks who were admitted to hospital because of threatening premature delivery (pains, hæmorrhage and/or rupture of the membranes) and were not delivered within 24 hours of admission, conservative therapy was successful in postponing delivery for at least two weeks in 30 per cent. A living child who survived the neonatal period was born in 74 per cent of the cases. Of 47 patients who were given allylestrenol as a routine measure delivery could be postponed in more than half and a living child was obtained in four fifths of the cases. Since the neonatal and late prognosis of small-size premature infants is poor, a continued search for possible means to prevent and arrest threatening premature labour should be made.

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markedly better than in the preceding period, living children were obtained in series A in about two thirds (16/24), in series B in one half (17/35) and in series C in nearly four fifths (28/34) of the cases.

Discussion

Attempts to prolong the pregnancy in cases in which premature delivery threatens are considered justified even if the membranes have already ruptured. It is true that maternal deaths have occurred but complications are uncommon and are rarely severe (Buemann and Lange 1962). Therapy should be directed to the cause but surgical treatment alone can be regarded as sufficient only in unmistakable cases of incompetence of the internal cervical os. In some such cases it has been possible to prove placental insufficiency and drug therapy possibly even including hormones has been recommended (Borglin 1962).

Results obtained in different institutions and even in the same institution during different periods cannot be regarded as directly comparable. In the 63 cases of Øvlisen and Iversen (1962) which were treated because of threatening abortion with 6α-methyl 17α acetoxy progesterone for one week delivery occurred in 27 cases while the treatment was in progress. In the series of Fuchs and Stakemann (1959) at the same institution treatment with progesterone or mere inactive oil produced similar results. Among Buemann's and Lange's 267 cases in which the estimated weight of the foetus was 1000–2500 g and the membranes had ruptured prematurely but delivery had not occurred within 24 hours the pregnancy continued for at least two weeks in 62 cases (= 22 per cent). Therapy was similar to that in series A and B of this study. The results were also similar but poorer than in our series C (53 per cent). In their study perinatal mortality was 28.8 per cent, and in the present study in series A and B together 33 per cent and in series C 17 per cent.

In series C of this study in which allylestrenol was used as a routine therapy successful postponement of labour was more frequent than in series A and B. The foetal prognosis was also better in series C if the patient was admitted to hospital between 25th-

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ECTOPIC KIDNEY AS A COMPLICATION OF PREGNANCY AND LABOUR

BY

ANNA BERGQVIST

An abnormal position of the kidney may be either congenital or acquired. From a nomenclature standpoint, these abnormal positions are distinguished by the congenital being called ectopies the acquired dystopies

Caspar Bauhin is said to have been the first to describe the congenital pelvic kidney during a lecture at an autopsy in 1599. Hohl in 1828 was the first to describe a patient with ectopic kidney which obstructed labour Since then isolated instances have been reported more or less regularly and the literature concerning investigations of frequency complication risks, and methods of treatment has grown. An American team of researchers Anderson Rice and Harris in 1949 collected a large series from the world literature It covers the period 1828- 948 In 1963 Notter Laurent, Abbas and Dufoix published a French series. The present paper is based mainly upon the works of these authors and upon a series of 30 case reports collected from the literature published since 1950 as well as those of two patients who were treated during 1964 in our department.

The incidence of ectopic kidney as judged from several large series of autopsies is one per thousand. Between 1/3 and 2/3 of ectopic kidneys are situated in the true pelvis. An American collection of 22,000 autopsies gave a frequency for ectopic pelvic kidney of 1/3142 (Thomas, Barton, 1936) In clinical observa

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The embryology of the kidney is intimately connected with that of the genital organs. The kidney begins to develop when the foetus measures 5 mm. Two protuberances from the Wolffian ducts appear at the level of S II and form the kidney tissue. An epithelial process on the back of the Wolffian duct and next to the cloaca connects with the kidney anlage to form the ureter and the kidney pelvis. The foetal kidney progresses cephalwards behind the Wolffian duct and will normally reach an abdominal position at the eighth week. During the upward progression of the kidney its bloodvessel supply changes. The kidney continues through several vessel areas and is successively supplied by these It can obtain temporary vessel supply from the sacral, hypogastric external iliac, common iliac arteries and from the aorta. The permanent vessel supply is first established when the kidneys are nearing their final position. This takes place when the foetus is 25–30 mm in length. During their upward progression the kidneys also move laterally and are rotated axially They do not reach their final position until the second year of life The superior extremity of the left kidney reaches T XI and the right kidney which lies somewhat lower reaches T XII

The ectopic kidney differs from the dystopic by having a short ureter and an abnormal vessel supply The temporary vessels become permanent when the upward progression of the kidney ceases The ectopic kidney is almost always axially rotated and is displaced medially The kidney often remains foetally lobulated and hypoplastic and embedded in a poorly-developed adipose capsule Ectopic kidneys are usually fixed and ordinarily cannot be displaced more than a few cm.

The reason for the faulty position has been discussed by many authors, but the problem is not yet solved. We find a greater frequency of genital malformations in patients with ectopic kidneys Frequency figures from 18–38 per cent are quoted. Uterine malformations are most common. Malformations of the pelvic bones are also fairly common.

The diagnosis of ectopic kidney is often first made at laparotomy The patient is operated on—because of pains or a palpable tumour—for suspected appendicitis, ectopic pregnancy or ovarian cyst About 80 per cent of the clinical diagnoses are first made at

tions the reported frequency varies considerably from 1-2 per thousand (Thomas Barton 1936) to 0 1 per thousand (Thompson Pace 1937) Many cases are not diagnosed during life therefore figures of about 0 1 per thousand are most common in clinical material Anderson *et al* report a frequency in pregnant women of about 1/5000—0.2 per thousand. No sex difference can be established. However the diagnosis is made more frequently in women because of vaginal examination

Dystopic kidneys are originally normal kidneys which have for one reason or another sunk downwards As they can be elevated from the pelvis they are very seldom a cause of obstetric dystocia. The position of the kidney in the pelvis predisposes to stasis in the urinary tract and can therefore give rise to infections or hydronephroses. If these cannot be cured by conservative therapy nephropexy is indicated. Cases have been described where a dystopic kidney has obstructed labour Manually raising the kidney is usually successful only in exceptional instances of dystopic kidney have Caesarean sections been necessary

Ectopic kidneys are quite another problem from the obstetric point of view They can be considered from two aspects according to their position according to the type of pelvic ectopic kidney anomaly

1 Lumbar ectopia a High = above L II
b Low = between the iliac crest and L III
c Iliolumbar = at iliac crest

2. Iliacal ectopia. The kidney is situated in iliac fossa.

3 Ilio-pelvic ectopia The kidney is situated in the pelvic inlet.

4 Pelvic ectopia The kidney is situated in the true pelvis

The ectopia can be either unilateral or bilateral Bilateral ectopic kidneys are often horseshoe kidneys the superior or inferior extremities being united. Sometimes one of the kidneys moves over to the other side both kidneys lying on the same side usually fused. Once in about 22 000 cases aplasia occurs on one side at the same time as ectopia occurs on the other If all the kidney tissue lies in the true pelvis, the treatment demands that special consideration be given to this aspect.

diagnosis during pregnancy is further complicated because X-ray examination should be avoided.

Pregnancy always predisposes to stasis in the urinary tract. Pelvic kidneys are especially exposed to this risk. Notter *et al.* report in their collected material that 15 per cent of the pregnant patients had kidney complications, the most common being pyelonephritis and hydronephrosis These conditions can usually be cured with conservative therapy in the form of antibiotics and possibly by drainage Nephrectomy is sometimes indicated. In five of the 98 patients reported by Anderson *et al.* nephrectomy was performed during pregnancy During labour the risk to the kidney increases. Usual signs of kidney damage are erythrocytes and albumen in the urine. To illustrate this, it can be mentioned that the last sign was used to diagnose ectopic kidney It is recommended that a tumour in the pelvis that is suspected of being an ectopic kidney be subjected to relatively strong pressure for a few minutes. If the tumour is an ectopic kidney albumen can then sometimes be found in the urine. In the ordinary course of events no more serious kidney damage occurs at delivery Anderson *et al.* had a complication rate of approximately 13 per cent and in the cases after 1950 the rate is 19 per cent. Most complications during labour leave no lasting damage to the kidney

Pregnancy can sometimes be influenced by the hypertensive effect of a diseased kidney Ectopic kidney can therefore cause hypertension or perhaps predispose to preeclampsia and prematurity Often, kidney complications first appear during labour The kidney can obstruct delivery either by its size and position or by causing a faulty position of the foetus. One or two deliveries may often go well, but a subsequent delivery may call for Caesarean section because of obstruction. This may be due to the fact that babies tend to be larger with increasing parity It may also be due to disease of the kidney The kidney may be fixed by an inflammatory process or enlarged by a hydronephrosis. Ectopic kidneys may also develop an acquired dystopia during pregnancy The kidney sinks lower and lower into the true pelvis with each pregnancy so that an obstruction in labour that earlier was merely relative now becomes absolute. When the kidney lies medially in the pelvis, the obstruction is often worse than when

Table I. *Time of Diagnosis in a Series of 91 Mothers.* Anderson *et al.* 1949.

Before pregnancy—approximately	5 %
During pregnancy—approximately	25 %
During labour—approximately	20 %
Immediate or late puerperium—approximately	9 %
Interval between pregnancies—approximately	9 %
Years after last pregnancy—approximately	25 %
At autopsy—approximately	8 %

operation The most common symptom of ectopic kidney is pain. This is often diffuse and uncharacteristic. It may be located in the lumbar region or in the legs Sometimes it mimics dysmenorrhoea Ectopic kidneys can also cause difficulties in evacuation and micturition These difficulties may occur even when the kidney is quite healthy but are more common in the pathological kidney The healthy ectopic kidney is more often free from symptoms. The position of the kidney predisposes to stasis in the urinary tract therefore the frequency of disease is 30–50 per cent higher in ectopic kidneys than otherwise Pyelonephritis, hydronephrosis and pyonephrosis are common in ectopic kidneys. Other kidney diseases are believed to occur with the same frequency as in normally situated kidneys. Because of the abnormal position ectopic kidney often gives a pathological picture difficult to interpret with atypical pain localization The diagnosis of a healthy ectopic kidney is difficult because the only sign is a palpable tumour An ectopic pelvic kidney cannot usually be palpated through the anterior abdominal wall but can generally be discovered at vaginal examination Unfortunately the diagnosis is almost always overlooked because ovarian tumours fibroids, and ectopic pregnancies are far commoner than ectopic pelvic kidneys therefore the diagnosis is not usually made preoperatively The diagnosis cannot normally be made before the first pregnancy because the patient seldom has any symptoms and thus does not visit the doctor Notter *et al* made the diagnosis in 3/45 patients before pregnancy Corresponding figures by Anderson *et al* can be found in Table I In the case reports from the period after 1950 the diagnosis was made before delivery in 16/46 during labour in 5/46 and after pregnancy in 25/46. The

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Table III. *Obstetric Methods Used in the Cases Reported by* Anderson *et al. and in Those Collected by the Author*

	ANDERSON et al.	Cases after 1950
Number of viable infants	209	32
Spontaneous delivery	153 (73 %)	16 (50 %)
foetal loss	13 (8 %)	3 (1 %)
Forceps + vacuum extractor	6	2
foetal loss	3	0
Breech presentation	1	4
foetal loss	5	2
Version + extraction	5	—
foetal loss	4	—
Caesarean section	32 (5 %)	9 (37 %)
foetal loss	3 (9 %)	1 (5 %)
Craniotomy		—

As can be seen from Table III the frequency of Caesarean section is considerably less in the report of Anderson *et al.* than in the later cases. Notter *et al.* in 92 deliveries had a Caesarean section rate of 28 per cent. It can also be seen that the foetal death rate with spontaneous delivery and with Caesarean section is about the same. Other obstetric methods such as forceps and version + extraction result in an alarming foetal death rate breech presentations have a mortality that is frightening.

The prognosis for the mother is good. Anderson *et al.* it is true report 10 deaths, but at the same time it must be remembered that these patients were treated before 1927. Notter *et al.* in their 45 case reports had one death during Caesarean section. In 32 cases after 1950 no deaths are reported. As Caesarean section is used increasingly to deliver patients with ectopic kidneys, the risk of this operation, although relatively slight, must none the less be taken into account.

The remote prognosis for the mother is on the whole more difficult to appraise. In approximately 15 per cent, renal complications occur some serious. Hydronephrosis and pyonephrosis not only endanger the kidney but also may give rise to hypertension, which may shorten the life of the mother. The threat to life, however is not alarming so long as there is one normal kidney properly situated. If all the kidney tissue lies in the true

it lies laterally Anderson *et al.* demonstrated with X-ray pictures a patient which they followed carefully during a spontaneous delivery The kidney occupied the lateral space in the pelvis and moved slowly upwards during labour The movement is, naturally impossible when the kidney is firmly fixed. Purely from the standpoint of figures more complications are reported in recent years. This however may be due to the fact that cases free from complications are not reported. Anderson *et al* report 73 per cent spontaneous deliveries. The corresponding figure by Notter *et al* is 58 per cent and in the reports after 1950 50 per cent. Among the complications occurring in the latter 18/56 were absolute obstructions. Cæsarean section was performed in 16 patients in the other two manual reposition of the kidney was successful, the deliveries progressing spontaneously In 9 patients malpresentations occurred 4 transverse, 4 breech and 1 face presentation. Here the situation was solved in 3 instances by Cæsarean section, in 3 others by external cephalic version and the remaining 3 were delivered spontaneously

A short report of the cases included in the report of Anderson *et al* and those that I collected from after 1950 are given in Table II in order to demonstrate the risk of complications which must be taken into account and the extent to which the more recent advances in the art of obstetrics affect the results.

As can be seen the percentage figures are practically identical. The only improvement is the reduced maternal mortality Anderson *et al* reported the death of 10 mothers. However these all occurred before 1927

Table II. *A Survey of the Report of Anderson et al and of the Cases after 1950 Collected by the Author*

	ANDERSON et al	Cases after 1950
Number of mothers	112	32
Number of maternal deaths	10	0
Number of pregnancies	~66	66
Number of abortions	47 (17.6 %)	10 (15.2 %)
Number of premature infants	9 (3.1 %)	4 (6.1 %)
Deaths of premature infants	5	4
Deaths of viable infants	35 (15.9 %)	9 (13.6 %)
Total foetal loss incl. abortions	82 (30.8 %)	19 (28.8 %)

Table IV *Time of Diagnosis in the Cases after 1950.*

Diagnostic Mode.	Before and during Pregnancy	During Labour	After Delivery
Caesarean section	0	5	1
foetal loss			
Spontaneous cephalic presentation	5	1	9
foetal loss	0	0	
Spontaneous breech presentation	1	—	3
foetal loss		—	2
Forceps + vacuum extraction		—	
foetal loss		—	0
Total number of cases	6	6	24
foetal loss	1	0	4

were lost—16 7 per cent. The prognosis for the child is thus improved by diagnosing ectopic kidney before or during labour

If a retroperitoneal tumour the size and form of a kidney is palpated in the pelvis an ectopic kidney ought to be suspected. Lovelady and Dockerty have published a series of approximately 100 extragenital tumours in the pelvis and of these almost 50 per cent were pelvic kidneys. The difficulty is to exclude ovarian tumours fibroids, and ectopic pregnancies. If the patient has or has had kidney disease and pregnancy can be excluded, urography should be carried out. During pregnancy suspicion of ectopic pregnancy or ovarian cyst can make it necessary to operate Coelioscopy permits good examination of the tumour and may save the patient from laparotomy If the tumour is discovered at the end of the pregnancy and the patient has had renal symptoms, urography may be justified. It is important, as indicated above for the diagnosis to be made before delivery With the active antenatal care practised nowadays this should present no difficulties. If a patient in good labour fails to progress a careful vaginal examination should be made in order to determine whether ectopic kidney can be excluded. If this is always done the treatment is no great problem. If a diagnosis is made before pregnancy the patient must report for regular urine examinations. There is

pelvis pregnancy and especially labour sometimes cause irreparable kidney damage. Under these circumstances therapeutic abortion and sterilization may be indicated if the first pergnancy introduces serious complications With a unilateral pelvic ectopic kidney these measures are not indicated. Sometimes when the kidney creates an absolute obstruction sterilization can be discussed after two or three Cæsarean sections. In general there is increasing difficulty for the child to pass the kidney with the number of pregnancies Notter *et al.* gave a complication rate among multiparæ of 61 3 per cent compared with an overall rate of 51 per cent.

The abortion frequency varies around 15 per cent. Ectopic kidney is said not to be responsible for abortions but the genital malformations that are often found at the same time can in isolated instances give an increased frequency The 10 abortions found in the material after 1950 occurred in 5 women. Two of these five had uterine anomalies (uterus arcuatus and uterus bicornis) One of the five had bacteriologically established toxoplasmosis Therefore with repeated abortions in connection with ectopic kidney hysterosalpingography should be performed in order to determine whether genital malformation can be excluded.

The frequency of premature birth is not increased in association with ectopic kidney but the prognosis for the child is still poor The perinatal mortality in the series of Anderson *et al.* is about 15 per cent, a figure which corresponds approximately to that of the cases after 1950 In the report of Anderson *et al* the mortality rate in children delivered by Cæsarean section was equally as great as in those delivered spontaneously In the material from the last decade the fœtal mortality is somewhat less with Cæsarean section At the same time Cæsarean section has increased from 15 per cent (Anderson *et al.* 1949) to 37 per cent (after 1950) A closer examination of the cases reported after 1950 reveals that the time at which the diagnosis is made has a prognostic importance *cf* Table IV

In 22 pregnancies the diagnosis was made before or during labour In these only one child was lost, at Cæsarean section performed because of abruptio placentæ However in 24 cases where the diagnosis remained unknown until after delivery 4 children

Table IV *Time of Diagnosis in the Cases after 1950.*

Diagnostic Mode	Before and during Pregnancy	During Labour	After Delivery
Caesarean section	9	5	1
foetal loss		0	
Spontaneous cephalic presentation	5		9
foetal loss	0	0	2
Spontaneous breech presentation	1	—	3
foetal loss		—	
Forceps + vacuum extraction		—	
foetal loss		—	0
Total number of cases	16	6	24
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Table V *A Survey of the Cases after 1950 Collected by the Author*

Author	Year	Age	Time of Diagnosis	Kidney Involved	Number of Pregnancies Abortions	Premature	Ful
14	1950	—	At delivery Obstructed labour	left	0	0	
21	1950	20	After delivery Urography	solitary	0	0	
34	1951	27	II-preg. Laparotomy Suspected ectopic pregnancy	left	0	0	
35	1952	20	After delivery Laparatomy	Horseshoe kidney	0	0	
7	1952	33	After IV pregn. Urography	right pelvic left lumbar	0	0	
7	1952	28	After pregn. Laparotomy Suspected ovarian cyst.	unilateral	0	0	
23	1952	29	During labour Obstructing tumour Urography	left	0	0	
15	1953	31	Before pregn. Urography	right pelvic left lumbar	0	0	
15	1953	25	Before pregn. Urography	right crossed fusion.	0	0	
15	1953	—	—	Horseshoe kidney	0	0	
15	1953	—	—	unilateral	0	0	
27	1955	32	After delivery I. Urography	bilateral with fusion.	1 (12th w)	0	
10	1955	29	Before pregn. Laparotomy	unilateral.	0	0	
38	1955	—	—	asymmetrical fusion.	0	0	
4	1955	41	After delivery II. Laparotomy Suspected ectopic pregn.	right pelvic left lumbar	1 (12th w)	0	
22	1956	34	During V pregn	left	0	1 (dead)	
30	1957	—	At delivery III	unilateral	0	0	
30	1957	—	At delivery I	left	0	0	
30	1957	—	After delivery III Laparotomy	left	0	0	
30	1957	—	After delivery III. Urography	left	0	(dead)	

...hod of ...very	Outcome Child	Mother	Remarks Follow-up.
...trean.	alive	alive	Albumen and erythrocytes in urine during l...
...c tumour disloc. upwards. ...hod's forceps			No kidney damage.
...rech. ...esarean.	dead	"	II-pregn. stone + abscess in ectopic kidney Ab... placentae. Nephrectomy
...c tumour disloc. upwards ...tan delivery	alive		No kidney damage.
...ntan.			Periodical pains in loins. No kidney damag...
...tan			Nephrectomy after pregnancy
...ic tumour disloc. upwards ...ntan delivery			During labour albumen and erythrocytes in... Kidney function after delivery normal.
...esarean			Short vagina. Small cervix. Obstruction.
...esarean			Short vagina. Small cervix. Obstruction.
...ntan.			Left hydronephrosis. —
...pontan. II Caesarean Caesarean			Kidney infections. Uterus arcuatus. Subtot... terectomy
Breech. II Transverse lie, ...esarean			Kidney infections during pregnancy. Uterus arcuatus.
...ech, Caesarean Caesarean II Caesarean			Bridge formation between the kidney pelv...
...ntan			Chronic constipation.
II, III, and V spontan			Bacteria, albumen, erythrocytes, leucocytes i... after V
...V external cephalic version	dead		
II spontan. III Caesarean	alive		Uterus bicornis. Hysterectomy
...esarean			Obstruction.
II spontan. III the kidney ...oc. upwards. Spontan			Left kidney hypoplastic.
II spontan			Left kidney deformed.
...transverse lie. Extraction	dead		

Author	Year	Age	Time of Diagnosis	Kidney Involved	Number of Pregnancies		
					Abortions	Premature	f.t
18	1958	~8	After IV-pregn. Urography	unilateral	3	1 (dead)	
8	1958	20	After delivery Laparotomy Susp ovarian cyst.	left	0	0	
11	1959	22	After I-pregn. Urography	right	0	0	
11	1959	51	After pregnancies Laparotomy Tumour	right	2	0	1
11	1959	31	Urography in connection with abort.	bilateral	1	0	1
13	1959	28	During delivery	Sigmoid kidney	0	0	
25	1960	24	During delivery	left	0	0	
~9	1960	35	During delivery II	left	0	0	
33	1960	27	During delivery	Solitary	0	0	
16	1962	27	At delivery IV	right	0	1 (dead)	
Own	1964	26	Pregn. I. Laparotomy Susp ectopic pregnancy	left	1 (12th w.) 1 (7th mon.)	0	
Own	1964	19	Before pregnancy Urography	right	0	0	

no indication to interrupt pregnancy associated with a unilateral ectopic kidney Possible infections are treated with drugs. Signs of preeclampsia and more serious infection indicate the need for admittance to hospital Where the entire kidney tissue of the patient is situated in the pelvis considerable care must be taken. Serious infection sometimes warrants interruption of pregnancy At the start of labour Caesarean section should usually be performed if the patient s entire kidney tissue lies in the danger zone If the ectopic kidney is unilateral spontaneous delivery should be attempted. If the head seems to pass spontaneously progress should be checked by repeated vaginal examinations. As long as the kidney is movable in the pelvis spontaneous delivery is possible However if the kidney is wedged between the head and the sacrum, Caesarean section should be carried out without undue delay

...y of	Outcome Child Mother	Remarks Follow-up
ro-Caesarean	alive	Nephrectomy in connection with Caesarean section.
	dead	Uterus unicornis. Left tube missing. Left ovary ectopic.
	alive	Normal function of both kidneys.
ion.		Normal genital organs
	—	Uterus bicornis. Spina bifida occulta.
rean.	alive	L V assimilated unilaterally to sacrum.
presentation. Caesarean		Normal genital organs.
sarean. II Caesarean		Nephropexy performed at 2nd Caesarean.
rean.		Albumen in urine after delivery
III, spontan.		Urinary infection during last pregnancy and after
Caesarean.	dead	delivery
High vacuum-extraction	alive	Toxoplasmosis at abort. Albumen and erythrocytes in urine during labour Normal genital organs.
ch		Urinary infection during pregn. and labour Erythrocytes in urine.

Nephrectomy is very rarely indicated and must never be performed if the other kidney is not functioning efficiently With breech presentations and transverse lies, external version should be attempted. Caesarean section is indicated if the kidney lies in the true pelvis and it cannot be pushed upwards, and if the breech presents because the foetal mortality is otherwise about 50 per cent. Under no circumstances must forceps be used to draw the head past the kidney Outlet forceps can be permitted, however if the kidney has risen out of the pelvis. In the puerperium, urine examinations and kidney function tests should be made, as well as kidney X-ray examination if this has not been made earlier

The principles of treatment can be summarized thus Ectopic kidney during pregnancy should be treated conservatively as far as possible With bilateral ectopic kidney usually Caesarean section should be performed. With unilateral pelvic ectopic kidney

spontaneous delivery is desirable if it is at all possible, because the kidney does not cause obstruction in 50 per cent of the cases. If there is an added indication for Cæsarean section—an elderly primigravida, preeclampsia breech presentation narrow pelvis—Cæsarean section should be considered even with a unilateral pelvic ectopic kidney With bilateral pelvic ectopic kidney kidney disease may justify both therapeutic abortion and sterilization.

SUMMARY

Three series reporting ectopic kidneys during pregnancy and labour are discussed one American from 1949 of 112 mothers one French from 1963 of 45 mothers one from the literature after 1950 of 30 mothers collected by the author as well as two cases from our department.

Nomenclature embryology and the symptoms of ectopic kidney are reviewed. The difficulty of diagnosis is discussed. In clinical material the diagnosis is not made before operation in 80 per cent of the cases. Kidney complications occur in pregnancy and labour in 15 per cent of the cases. Fifty per cent of the deliveries are uncomplicated. The maternal mortality is low The infant mortality is still 15 per cent. We find that when ectopic kidney is diagnosed before or during delivery the mortality of the children is considerably lower than when the condition remains undiagnosed.

The treatment of ectopic kidney during pregnancy should as far as possible be conservative With a bilateral pelvic ectopic kidney usually Cæsarean section should be performed with a unilateral abnormality spontaneous delivery should be attempted. With additional complications Cæsarean section should be considered.

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OBSTETRIC STUDIES IN WOMEN WITH HISTORIES OF URINARY INFECTIONS

BY

CARL FELDING

Improvement in techniques for the diagnosis of urinary infections has led to an increased awareness of the occurrence and effects of such infections in childhood and in women of childbearing age. More recently obstetricians have developed an interest in the influence of urinary infections preceding pregnancy on the course of subsequent deliveries.

The present work consists of an obstetric evaluation of 259 women with histories of urinary infections The series comprises 421 deliveries including 5 twin deliveries in which the incidence of toxæmia prematurity and perinatal mortality has been assessed.

Literature

The problem of chronic pyelonephritis its significance and place in modern medicine, is convincingly illustrated by Raaschou (1948 and 1957) who demonstrated from autopsy material that

1 5.6 per cent of the patients in the autopsy series died with or from chronic pyelonephritis

2 63 per cent of all uræmic patients suffered from chronic pyelonephritis

3 the primary form *i e* without obstruction in the efferent urinary passages was twice as common in females as in males

4 the disease was most frequently undiagnosed during life

5 the presence of objective symptoms frequently varied and

6. urography shows normal findings in 20 per cent and renal biopsy may also reveal normal findings

So-called toxemias of pregnancy may on further investigation, be demonstrated to be cases of chronic pyelonephritis. By means of prolonged follow-up investigations Hochuli and Käser (1958) demonstrated that among 104 patients with severe pre-eclampsia there were 17 with chronic pyelonephritis. Finnerty (1956) Bucht *et al* (1960) and Milliez *et al.* (1949) were also of the same opinion.

Kass (1956) showed that among 152 pregnant women at term, 11 per cent had asymptomatic bacilluria and the same author (1960) demonstrated increased foetal mortality in patients with untreated bacilluria during pregnancy as compared with a group who received treatment. The difference was convincing but the numbers were limited (48 and 43 patients, respectively)

The works of greatest interest in this connection are, however those of Rauramo *et al* (1962) and Mackay (1963) who describe pregnancies in women in whom pyelonephritis had been diagnosed prior to the commencement of the pregnancy Rauramos material originates from 1930–57 and includes patients with both nephritis and pyelonephritis. In the text, the combined results are given for both conditions e g. where the infantile prognosis is concerned but the author concludes from his 236 patients with pyelonephritis that the risk of developing toxemia of pregnancy is 14.6 per cent for women who conceive within three years of the onset of renal disease but only 3.3 per cent if a longer interval has elapsed.

Mackays material consisting of patients with chronic pyelonephritis originates from the years 1951–1960 and comprises 33 cases of which three had renal tuberculosis. He defines the criteria for diagnosis and the radiographic findings and records foetal death in eight cases out of the 33 pregnancies Four patients had initial blood urea values of over 50 mg per cent. None of these women had living infants.

The author s material

From an extensive series consisting of women with various

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2 63 per cent of all uræmic patients suffered from chronic pyelonephritis
3. the primary form *i e* without obstruction in the efferent urinary passages was twice as common in females as in males,
4 the disease was most frequently undiagnosed during life
5. the presence of objective symptoms frequently varied and

demonstrable reduction in renal function or significant radiological changes

It must, however be admitted that the boundaries between these groups are not sharp and that with better diagnostic techniques a somewhat different diagnostic subdivision might have been obtained.

Bacterial counts were not undertaken. The quantitative estimates consists of copious moderate and scanty growth.

Renal biopsy was not undertaken as a routine. In 26 cases, however the results of pathological investigation of the kidney from nephrectomy biopsy or autopsy are available.

Age distribution. Seventy five patients were under 15 years, 175 were 15–30 years and 9 were 30–40 years at the time of the presumed onset of the renal disease Thus, none of the patients were over the age of 40 years.

Two hundred and fourteen of the patients were nulligravidæ when the diagnosis was established and 45 were gravidæ 1–5.

Radiographic findings. Great emphasis was laid on urography and straight X ray of the renal regions. Out of 180 urographic series 139 were re-examined and the findings revised, if necessary The criteria which are considered to be of importance in the radiographic assessment (Sven Johansson, 1963) are the size of the kidney both the actual size and in relation to the contralateral organ, the relationship between cortex and medulla, signs of shrinkage the appearance of the papillæ, the morphology of the renal pelvis and the ureters, calcifications, the excretory conditions and symmetry The patients were not investigated routinely for ureteric reflux. The radiological diagnosis is mainly based on the focal form of the disease (Hodson 1959)

Seventy two out of the 180 urographic studies were found to be pathological. In certain patients, several pathological conditions were encountered simultaneously The following may be mentioned among the more important diagnoses hydronephrosis in 16 cases calculi in 19 cases papillary necrosis in 12 cases renal hypoplasia in 9 cases congenital abnormalities in 20 cases and medullary sponge kidney in two cases.

Surgical intervention. Nephrectomy was performed in 23 patients, and uretero- or pyelolithotomy in four cases. Further

renal diseases a total of 259 patients with urinary infections were found, all of whom had at least one delivery

These patients had the following common features

1 The urinary infection had been diagnosed and treated in hospital
2 The urinary infection occurred before the patient became pregnant Pyelonephritis of pregnancy was not included.
3 With two exceptions all of the 421 deliveries occurred in hospital
4 The case histories from the hospitals concerned were analysed both as regards the renal disease and the delivery
5 The ages of the patients at the onset of the renal disease were between 0 and 40 years
6 Diabetic patients are not included

Diagnostic criteria

Sixty-eight of the cases fulfil the criteria laid down by Bucht *et al* (1960) for establishing the diagnosis of chronic pyelonephritis These include three out of the following six features

1 History of attacks of cystitis and/or pyelonephritis
2 Microscopic examination of the urine reveals numerous white blood cells + renal epithelium
3 Definite bacteruria *i e* more than 100 000 bacteria per ml. morning urine
4 Positive pathological findings in a biopsy [(or nephrectomy or autopsy material) Author s addition]
5 Reduced renal function
6 Positive radiographic findings

Where three of the above-mentioned features are present, the diagnosis is relatively certain

One hundred and thirty-four of the patients had well defined episodes of pyrexia pyuria bacteruria, lumbar pain renal tenderness and subjective renal discomfort but no signs of reduced renal function or gross radiological changes. The remainder *i.e* 57 patients had had pyuria but without definite information about pyrexia. The majority had however raised ESRs (more than 20 mm) lumbar pain and/or recurrent symptoms but no

plastic procedures were carried out in the renal pelvis and operative treatment of aberrant renal vessels was sometimes performed. Diverse interventions via the urethra were also carried out on a lesser scale.

Renal function This is illustrated, *inter alia* by the ability to concentrate. In 72 cases, information on this was available. Twenty-one of these concentrated urine to a maximal specific gravity of 1020 The pitressin test was not carried out as a routine nor was estimation of molarity In 11 cases, initially raised residual nitrogen and reduced creatinine excretion were present. During the period concerned, this investigation was carried out mainly by means of the creatinine clearance test.

Obstetric studies

The 259 women in the series had a total of 421 deliveries, 5 of which were twin deliveries = 426 infants. In this investigation, abortions, extrauterine or other pregnancies were not included. The difference between late abortions and early deliveries was defined, at the time during which the material was collected, thus A stillborn foetus of less than 35 cm (13 ½") is classified as an abortion while a stillborn foetus of over 35 cm is classified as an infant and its birth as a delivery A liveborn foetus is classified as an infant irrespective of the birth weight.

Only the deliveries which occurred after the renal disease had been diagnosed are included in the investigation.

Parity

139 patients each had	1 delivery	= 139 deliveries
85	2 deliveries	= 170
28	3	= 84
7	4	= 28
259		421

Infant prognosis. The total infant mortality was 18 deaths out of 426 = 41.8 ‰ (revised for twins 40.3 ‰) Causes of death Prematurity in 8 cases, ante-partum foetal death (FMAP) in 3 cases, aspiration + asphyxia in 2 cases, erythroblastosis foetalis

Table I. *Fifteen Women with 18 Dead Infants. Foetal Weight. Causes of Foetal Death. Subsequent Pregnancies*

Group	Patient	Year	Complications	Foetal weight	Cause of death	Subsequent pregnancies
Group I	AO 1919	1941	Severe toxemia	1310	FMAP (foetus mort. ante partum)	1955 spontaneous abortion
	EB 1937	1950	Toxoplasmosis(?)	2220	Asphyxia	1960 living twins
	HA 1911	1939	Severe toxemia. Twins	870 (Tw nr 2)	Immaturity	1st twin alive Mother died 1943 uremia
	HN 1924	1944	Severe toxemia	740	Immaturity	1949 premature labour dead infant 1730 gr
	IN 1916	1953	Mild toxemia	2380	FMAP	1955, a normal delivery
	RA 1930	1953	Rh immunisation	4310	Erythroblastosis foetalis	1955, erythroblastosis foetalis dead infant 1958 living Rh neg. infant
	EK 1917	1938	Severe toxemia	950	Immaturity	1939 living infant
Group II	GS 1935	1959	Syphilis	1900	Malformations	1962 FMAP
	BO 1922	1944	Premature labour	1650	Immaturity	1948 and 1949 2 normal deliveries
	IA 1929	1954				
	KA 1940	1960	"	870		
	IP 1920	1944	Mild toxemia	1490		
	EG 1919	1945	Complication of umbilical cord	2840	Aspiration	1946–50 3 normal deliveries
	EJ 1933	1961	Premature labour	3810	Asphyxia	1951 living infant
	II 1933	1956	Complication of umbilical cord	1300	Immaturity	1954–57 2 normal deliveries
				3000	Asphyxia	1950–1958 normal deliveries

in 2 cases, complications pertaining to the cord in 2 cases and multiple malformations in one case

The birth weight was less than 2500 g (5 ½ lbs) in 34 out of 426 = 79.8 ‰. This figure includes 14 of the perinatal deaths.

Maternal prognosis. Dieckmann's subdivision of toxemia of pregnancy (1952) is employed for classification of pre-eclamptic toxemia into mild or severe degrees. Thirteen patients with severe pre-eclamptic toxemia are concerned here, one of whom had a recurrence during a subsequent pregnancy. In addition, among these thirteen cases there was one case of eclampsia. No maternal deaths occurred although it is known that at least four patients died from the renal disease some years after delivery. The incidence of pre-eclamptic toxemia (severe according to Dieckmann) including one case of eclampsia was thus 13 out of 421 deliveries = 3 per cent.

It is, unfortunately, impossible to compare this series with a normal series as classification during the period in question (25 years) has varied and because the material originates from many different clinics. It is, however, apparent that there was a considerable difference in the incidence of toxemia between the 68 patients who fulfiller the criteria for chronic pyelonephritis (termed here Group 1) and the remaining cases (Group 2) (Table II.)

Four of these patients with severe toxemia of pregnancy had at least one further delivery but recurrence of the toxemia occurred in one case only. This is in agreement with the observation made by Mackay (1963).

Urinary infections during the puerperium. In 57 patients urinary infections were encountered during the puerperium. This is, however, a minimum figure as routine examinations of the urine for bacteriuria and pyuria were not undertaken on all the patients. Among 21 patients with subsequent deliveries, recurrence was encountered in 8. Definite figures for urinary infections in the puerperium are difficult to obtain but, in this series, the majority were found in Group I.

In evaluating factors such as the perinatal mortality, prematurity and the toxemias of pregnancy, it appears reasonable to subdivide the material into two groups. The first group con-

Table II. *Thirteen Women with Severe Toxæmia. Result of Delivery Fœtal Weight and Subsequent Pregnancies*

	Patien		Year of Delivery	State of Infant	Fœtal Weight		Subsequent Pregnancies
Group I	GN	1922	1950	alive	3930		0
	BC	1914	1945,	alive	4370		0 mother died 1955, chronic pyelonephritis
	IP	1919	1948	alive	2450		0
	HJ	1919	1952	alive	1860		0 mother died 1953 uræmia
	OJ	1903	1938	alive	1860		0
	IM	1913	1943,	alive	2360		therapeutic abortion 1943
	HA	1911	1939,	twins	1600	alive	0
					870	dead	mother died 1943, uræmia
	EK	1917	1938	dead	950		1939 severe toxæmia Living infant. Mother died 1945, renal disease
	AO	1919	1941	FMAP	1310		1955, spontaneous abortion
	HN	1924	1944,	FMAP	740		1949 delivery of a dead infant 1730 Therapeutic abortion and tubal ligation 1951
Group II	SL	1937	1955	alive	4070		1957 and 1961 normal deliveries
	EL	1932	1952	alive	4320		1955 therapeutic abortion. 1957 normal delivery
	SW	1922	1954,	alive	4250		0

Table IV *The Incidence of Severe Pre-eclamptic Toxaemia Perinatal Mortality Number of Live Births of Infants Weighing Less than 2500 g (5 ½ lbs) and the Incidence of Urinary Infection in the Puerperium in the Total Material and in Groups 1 and 2 respectively*

	Severe Pre-eclampsia %	Perinatal Mortality ‰	Living Infants < 2500 g %	Urinary Infection in Puerperium %
Total	3	41.8	4.7	13.5
Group I	14.7	86	10	32
Group II	1.6	27.9	3.4	7.8

sists of the 68 patients who fulfil the criteria established by Bucht *et al.* (1960) for the diagnosis of chronic pyelonephritis. The second group consists of the remaining 191 patients with well-defined acute urinary infections without demonstrable radiographic changes in the kidneys or reduced renal function.

Table I details the number of infants who died in the perinatal period, the causes of death and the complications which occurred during pregnancy and delivery in the two groups. In Group I, a total of 9 infant deaths is found out of a total of 104 infants, i.e. 86 ‰. Further it is evident that severe pre-eclamptic toxaemia complicated the delivery in four cases. In Group II the perinatal mortality was 9 out of 322 infants = 27.9 ‰. In this group no cases of severe pre-eclamptic toxaemia occurred.

Table II shows the distribution of severe pre-eclamptic toxaemia (including here the only case of eclampsia in the series). In Group I there were 10 cases out of 68 patients = 14.7 per cent. One patient had severe pre-eclamptic toxaemia also during a subsequent pregnancy and four women died from the renal disease within 4 to 7 years after delivery.

In Group II there were 2 women out of 191 with severe pre-eclamptic toxaemia and one case of eclampsia, i.e. 3 out of 191 = 1.6 per cent. There were no maternal deaths in this group during the period of observation.

Table III shows the liveborn infants with birth weights of under 2500 g (5 ½ lbs) and the associated circumstances at birth. A high incidence of toxaemia is shown in Group I (5 out of 9)

Table III. *Twenty Women Delivered from a Living Infant Weight under 2500 Complications of Delivery and Result of Subsequent Pregnancies*

	Patient		Year of delivery Complications		Fetal weight	Subsequent pregnancies
Group I	IP	1919	1948	severe toxemia	2450	o mother died 1953 uremia
	LP	1909	1952	premature labour	2460	o
	LB	1933	1952		1840	o
	HJ	1919	1952	severe toxemia	1860	o
	KM	1916	1951	twins	1820 2060	o
	OJ	1903	1938	severe toxemia	1860	o
	AK	1924	1953,	premature labour	2400	o
	JM	1913	1943	severe toxemia	2360	therapeutic abortion 1943
	HA	1911	1939	" (twins)	1600	Twin nr 2 (870 gr) died. No further pregnancies. Mother died 1943, uremia
Group II	GM	1923	1951	premature labour	1550	o
	IH	1921	1953,		1730	o
	KG	1924	1956		2020	o
	SN	1918	1957		1930	o
	KN	1938	1956		1700	1961 normal delivery weight of infant 2830
	KB	1938	962		2000	o
	MN	193	1958		2360	1961 a normal delivery weight 2640
	SL	1934	1956		2260	1961 a norm l delivery weight 3050
	GI	1934	952		1850	o
	IR	1931	1963,		2400	(cancer colli uteri)
	BN	1931	1950	"	2420	1952 nd 957 ormal deliveries

developing toxemia of pregnancy and the interval between the onset of renal disease and the delivery This may hold true for the acute renal diseases. It is however frequently impossible to determine when a chronic pyelonephritis commenced and, for this reason it is not possible to determine the interval.

The present series shows that the 68 patients who fulfil the criteria for the diagnosis of chronic pyelonephritis have considerably poorer obstetric prognoses than the 191 patients who are classified as acute pyelonephritis or uncomplicated urinary infections. This holds also for prematurity perinatal mortality the toxemias of pregnancy and urinary infections during the puerperium.

The material illustrates the influence of the renal disease on pregnancy and delivery On the other hand, no attempt has been made to assess the influence of the pregnancy on the renal disease. Such a task is outside the scope of the present investigation because it requires, among other things knowledge of the course of the chronic pyelonephritis in the individual cases

SUMMARY

The present investigation is an assessment of the obstetric prognosis in women with histories of urinary infections. The series consists of 259 women who had borne 426 infants All of the patients were under the age of 40 years at the onset of the renal disease and in all cases the onset of the renal disease was unconnected with pregnancy The material did not include any diabetic patients.

The series is subdivided into two groups, the first of which comprises 68 women who fulfil the criteria defined for the diagnosis of chronic pyelonephritis The second group consists of 191 patients with acute urinary infections. The obstetrical prognosis is expressed as 1 severe pre-eclamptic toxemia 2. perinatal mortality 3 liveborn infants weighing less than 2500 g (5 ½ lbs) and 4. urinary infections during the puerperium. The percentage incidences of these conditions in Group 1 were 14 7 8.6 10 and 32 and the corresponding percentages for Group 2 were 1 6 2 7 3.4 and 7.8

while toxæmia did not occur as an accompanying or precipitating factor in Group II

Table IV gives a tabular presentation of the above-mentioned conditions and also shows the percentage distribution of urinary infections in the puerperium.

Discussion

Reports of investigations concerning women with urinary infections correlated with subsequent pregnancies and deliveries are relatively scanty in the literature despite the numerous publications on pyelonephritis during the past decades The investigations available are not always easy to compare on account of the different diagnostic criteria employed in the definitions of pyelonephritis and the toxæmias of pregnancy

The material presented here is based mainly upon a review of the case histories of a 25 years series from 1932–1956 from a medical department and for obvious reasons the investigations undertaken are not up to date When, however an obstetrician attempts to assess the situation of a pregnant woman and her obstetric prognosis he is excluded from nephrological investigation with urography renal biopsy etc., and he can only evalute the patient on the basis of the information available from the time before the pregnancy Thus review of material as in this investigation appears to be justified.

It may be impossible to determine the extent to which the current criteria of proteinuria oedema and increase in blood pressure in a pregnant woman with renal disease represent a so-called toxæmia of pregnancy or aggravation of the pre-existing renal disease In the present series the cases have been evaluated thus Where the findings remained unchanged throughout pregnancy delivery and the puerperium the case was not classified as a toxæmia of pregnancy On the other hand, where a transient increase of the above-mentioned signs occurred the case was classified as toxæmia. Only by means of repeated renal biopsies, it is possible to elucidate the problem further (Pollak V and Kark, R. 196)

Rauramo (1962) found a relationship between the risk of

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A CASE OF MASCULINE PSEUDOHERMAPHRODITISM REPRESENTING GOLDBERG AND MAXWELL SYNDROME (TESTICULAR FEMINIZATION)

BY

SIMO LAURILA

Hermaphroditism has been known to humanity from time immemorial, and the medical literature contains abundant descriptions of it. Medical science uses the term hermaphroditism when the gonads of an individual are found to have both ovarian and testicular tissue, usually in such a way that one gonad is an ovary and the other a testis. These patients manifest, for obvious reasons, both masculine and feminine features. The term pseudohermaphroditism is applied in those cases of intersexuality in which the gonad structure is uniform, corresponding to one sex or the other but in which divergencies from the sex in question occur in the structure of the external organs of generation and in the development of secondary sexual characters. The anomalous development of the external genitals does in fact reveal intersexuality in the majority of cases. Sometimes pseudohermaphroditism occurs in such a form that the external sexual characters correspond almost exactly to one sex, but to the opposite one from what the structure of the gonads would imply In such cases the making of a diagnosis is difficult, and it can generally be done only in connection with an operation or an autopsy Goldberg and Maxwell (1948) as well as Schneider van Ommen and Hoerr (1952) described, on the basis of a total of 20 cases a pseudohermaphroditismus masculinus syndrome representing this latter type Many such cases have been reported sub-

A considerable difference is thus demonstrated in the obstetric prognosis between the two groups in favour of the acute urinary infections which do not appear to influence the course and the result of the delivery to any great extent.

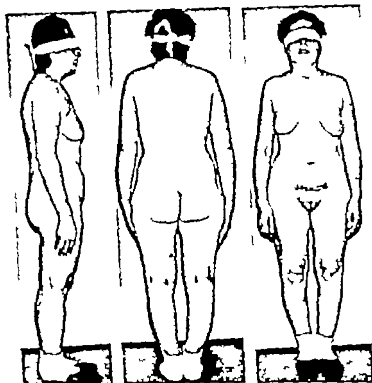
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Fig

The patient had been born at term. At 6 she fell ill with scarlet fever which passed without leaving any complications. During the last years she had frequently had bacterial infection of the urinary tract, but she gave no history of any other diseases. At 14 the mammary glands of the patient began to grow and she remembered having grown fast at the age of 5–16. In spite of having attained the age of puberty she had no menstruation. However she felt herself to be woman and experienced sexual desire towards the male sex. She married at the age of 25. Before that, in connection with gynaecological examination, the patient had been told, that she could not have any children, on account of developmental disorders of the uterus. None the less her conjugal lif was unimpeachable and the patient f lt deep attachment to her husband from the very first. Frigidity did not occur.

The patient knew of no history of pathological amenorrhea in the women of her family. Her elder sister and three brothers were healthy and had

sequently in the medical literature Morris (1953) collected 82 cases from the literature and 6 additional cases were reported by Hauser and associates (1957) In this paper we restate the features of this syndrome and describe a further case investigated in detail which fulfils the necessary criteria.

The patients reported by Goldberg and Maxwell had a fully feminine outward habitus The mammary glands were well developed the areolæ and the nipples somewhat smaller than normal The external genitals were quite feminine the labia generally slightly hypoplastic The pubic and axillary hair was absent or very scanty Vaginal examination revealed a narrow and short vagina terminating in an atretic sac. Its floor sometimes showed a small fold of mucous membrane in the place corresponding to the portio On bimanual examination neither the cervix nor the fundus of the uterus could be palpated, a finding which explains the amenorrhoea in these patients. Frequently the gonads could not be palpated either

At operation or autopsy the findings are complete uterine aplasia or small rudimentary parts of the uterus. The gonads are small situated usually intra abdominally but less frequently inside inguinal hernias as in cases of Ward Mc Quaid and Lennon (1950) and Beatty Champ and Swyer (1953) In specimens taken from the gonads microscopic examination reveals immature testicular structure without spermatogenesis. Ovarian tissue is entirely absent. The syndrome frequently has a familial occurrence Thus in the series reported by Schneider and associates there were 6 such cases in three successive generations of the same family

Case report

The patient (Record no 2089/I-59) (Fig 1) was at the time of first admission in September 959 a married woman of 39. She had attended a gynaecologist in her home town because of repeated urinary infections. Urography then showed pelvis duplex in the left kidney which finding was interpreted to be related to the recurring bacterial infection of the urinary tract from which the patient suffered. Because of the patient s primary amenorrhoea and uterine aplasia revealed by the gynaecological examination she was referred to the First Women s Clinic of the University of Helsinki for detailed examination.

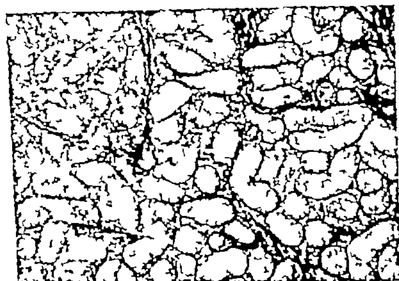


Fig. 2

children On the other hand, her younger sister was affected with the same symptoms as the patient herself such as primary amenorrhoea and absence of axillary and pubic hair This sister had also been told in connection with previous gynaecological examination that she could not have any children. In spite of repeated persuasion we were unfortunately unable to induce her to come up for an examination

The constitution of the patient examined by us was of the thletic type, rawboned, height 66 cm, weight 83 kg She had the appearance of a fairly normal robust woman her gestures, expression and behaviour were womanly The axillary and pubic hair was absent The mammary glands were large The external genitals were completely feminine, the labia somewhat hypoplastic the clitoris of normal size Internal examination showed that the vagina was comparatively narrow 8.5 cm long closed sac with no portio visible on its floor Slight folds, sort of rugae formation, were found on the vaginal walls Bimanual palpation did not reveal any parts of the uterus nor any other internal organs of generation

Roentgenological examination of the kidneys revealed double pelvis in the left kidney but no other deviation from the normal X-ray examination of the cranium showed slight osteoporosis, the sella turcica had a normal size and shape No bone anomalies were found in the bones of the spinal column, the pelvis or the fingers Vaginal smears revealed a moderate oestrogen effect The twenty-four hour excretion into the urine of gonadotrophic

Fig 2a



Fig 2b

the testes and the cells found in them were Sertoli cells. No spermatogenesis could be found in the testicular canals. The connective tissue surrounding the ducts revealed in places yellowish cells, comparatively large and rich in cytoplasm, forming islets. These were obviously Leydig cells. No ovarian tissue could be found in the samples. (Figs. 2 a, b and c) The sex chromatin of the patient was studied on a sample taken from the skin. It did not show any features characteristic of a female. The sex chromatin examination of blood cells indicated rather masculine type Only connective tissue scarce in cells was found at biopsy of a mammary gland and no glandular tissue was seen.

Discussion

In the patient described here we found all those features which are characteristic of the masculine pseudohermaphroditism type reported by Goldberg and Maxwell primary amenorrhoea, a completely feminine outward habitus absence of axillary and pubic hair rudimentary uterus and hypoplastic intra abdominal testes, in which no ovarian tissue could be found.

The gonads of the patient secrete fairly abundant amounts of oestrogenic hormones as judged by vaginal smears and the determination of the excretion of hormones in the urine. A further indication of the abundant oestrogen secretion of these hypoplastic testes is provided as well by the report of Goldberg and Maxwell of a patient who after castration exhibited typical climacteric symptoms, while the oestrogenic effect disappeared in vaginal smears.

Correct clinical diagnosis in these patients is difficult. Determination of sex chromatin is of benefit but verification of the diagnosis is only obtained by biopsy specimens from the gonads. A probable diagnosis can, however be established without laparatomy if the syndrome described above is borne in mind.

SUMMARY

The clinical features of Goldberg and Maxwell syndrome are described. A new case of this syndrome is reported.

Table I

	Urinary Gonadotrophin (mouse units/24 hrs)	Urinary Oestrogen (mouse units/24 hrs)	Urinary 17-ketosteroids (mg/24 hrs)
First determination	> 40	75	8.7
Second determination	> 40	50	14.1
Third determination	> 40	100	–

hormones, oestrogens and 17-ketosteroids was determined three times prior to operation. The results are seen in Table I. Routine examinations of the blood and the urine yielded no results deviating from the normal: the Wassermann and Kahn tests were negative; the sedimentation rate was 5 mm/h, haemoglobin 14.3 g, erythrocyte count 4,480,000, colour index 0.99, leukocyte count of the blood 6800 (eosinophil and basophil cells 0 %, transitional leukocytes 1.0 %, polymorphonuclear cells 74.0 %, lymphocytes 25.0 %, monocytes 0 %); non-protein nitrogen 35 mg%; urinary sediment normal.

On the basis of the examination the following diagnosis was made: Rudimentary bicornute uterus. It was in fact found at operation that this diagnosis was correct, although no case of simple gynatresia was involved.

With a view to obtaining an accurate knowledge of the internal genitals and in order to bring about possible fertility, laparotomy was resorted to (Professor Turunen). At first no internal genitals could be found, but the inner surface of the pelvis was lined with smooth peritoneal surfaces. Finally small gonads with smooth surfaces were found high above the linea terminalis. The right gonad was approximately the size of the terminal phalanx of the little finger, the left one was somewhat larger. On the caudal parts of the gonads there was on either side a small rudimentary uterine bud, and a comparatively well-developed round ligament. On the lateral sides of the gonads there were tubal rudiments about 2 cm long, terminating in atretic sacs and showing some fimbriae. The gonads were incised in the longitudinal direction up to the hilus and thin specimens were taken from the entire incision surface for microscopic examination. Large yellowish, solid areas were seen on the incision surface and around them smaller greyish areas which apparently had some small cysts. The wounds were sutured, since no corrective surgery could be contemplated.

The microscopic examination of the specimens taken from the gonads showed that the gonads were indisputably hypoplastic testicular tissue (Professor v. Numers). The specimens revealed mainly hyalinized thick connective tissue, but ducts formed by epithelial cells were also present. In some of them a small lumen could be observed in the middle. For the most part the ducts were filled with epithelial cells. These varied slightly in structure: some were more abundant in cytoplasm, and in some cases the shapes were more or less star like. The ducts in question were without doubt glandular ducts of

Knutsson, F. Rybo G. and Ahberg A. Acta obst. et gynec. scandinav. 44, 323, 1965

From the Department of Obstetrics and Gynecology (Professor Sam Brody) and the Department of Pathology (Professor Jan Mellgren) University of Gothenburg, Sweden

ANOVULATORY EFFECT OF LYNESTRENOL IN COMBINATION WITH MESTRANOL

BY

F. KNUTSSON, G. RYBO AND Å. AHBERG

Progesterone was successfully extracted and isolated from pigs ovaries for the first time in 1934 and was also synthesized in the same year. It had long been believed that the secretion from the corpus luteum was responsible for inhibiting ovulation and suppressing oestrus and in 1937 it was finally proved (Makepeace Weinstein and Friedman, 1937) that ovulation in rabbits could be prevented with progesterone. This anovulatory effect of progesterone has since been demonstrated in other animals also such as the rat guinea-pig, mouse sheep and cow.

The first orally active progesterone derivative anhydrohydroxy progesterone or ethisterone was synthesized in the late 1930 s. Since then a number of orally administered progesterone and testosterone derivatives of greater or lesser effectiveness have been produced. These derivatives, all of which have similar effects to progesterone have been given the name of gestagens or progestagens (Marjerek et al. 1960). As early as 1953–1954 Tullner and Hertz (1953) showed in animal experiments that 19-norprogesterone was more active than progesterone and that the 19-nortestosterone derivatives were more active than the corresponding testosterone derivatives. During the years that followed a number of papers describing animal experiments were published (Hertz et al. 1954 Slechta et al. 1954 Pincus et al. 1956 I II and Saunders et al. 1957) all of which pointed to the progestagenic effect of these steroids and to the greater activity of the 19-nortestosterone derivatives.

During the same period results of tests on humans involving

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Material and Methods

The series comprises ten women in the age group 39–49 one was 39 years old, four were 42 four were 43 and one was 49. All the patients had an uterus enlarged by myomata and displayed such clinical symptoms as to constitute grounds for surgical treat ment. They stated that their menstrual periods had always been regular

The patients were observed through two successive cycles. The first of these was a control cycle without administration of hor mone while during the second cycle 2.5 mg lynestrenol was given daily in combination with 75 γ mestranol from the 5th to the 24th days of the cycle. As far as possible the patients were operated on the 25th day of the treatment cycle although in a few cases the operation could not be performed until the 26th or 27th day

The following data were recorded during both the control cycle and the treatment cycle basal temperature excretion of pregnanediol in urine on the 6th–7th, 13th–14th, 19th–20th and 23rd–24th days the degree of arborisation of the cervical secretion on the 8th and 21st days and the histological appearance of the endometrium on the 25th day GOT GPT alkaline phosphatases, serum bilirubin and thymol reaction were determined once during the control cycle and on the 8th and 24th days of the treatment cycle A complete record of side effects was kept during the treat ment cycle

At the time of operation a careful inspection was made of the ovaries. When no typical fresh corpus luteum was found the larger of the two ovaries was removed for histological examina tion. In one case both ovaries were taken, while in two cases resections only were made from cystic areas in the ovaries. In addition, four complete ovaries with fresh corpora lutea from four untreated women in the same age group and with the same clinical diagnosis and grounds for operation were made available as control material. Three of these women had undergone opera tion on the 25th day of their cycles and one on the 17th day

Pregnanediol in the urine was determined by the method of Klopper et al. (1955) as modified by Gemzell et al. (1958) GOT GPT and other hepatic function tests were performed as

oral administration of these steroids were published (Ferin, 1956 Greenblatt, 1956 Rock *et al*, 1956 Ferin, 1957 Rock *et al*. 1957 Ferin and Vanek, 1958 Gansewinkel *et al* 1958 Pincus *et al*., 1958 and Matsumoto *et al*. 1960) The preparations administered in these series also proved to be potent progestagens the 19-nor-derivatives being the most effective. They had a reliable anovulatory effect without androgenic properties. The patients reverted to normal ovulatory menstrual cycles after the drugs were discontinued.

In 1959 de Winter *et al*. reported on a new group of steroids, *3-desoxo-19 nortestosterones known as estrenols*. The ethinyl and allyl derivatives of estrenol have proved to possess valuable progestagenic properties (Kopera and Huix, 1962) In animal experiments by Overbeck *et al* (1962) 17 β-hydroxy 17 α-ethinyl-estr 4-en (generic name lynestrenol) showed itself to have a reliable inhibiting effect on ovulation which effect was greatly potentiated by the addition of oestrogen Lynestrenol has proved *also to be an effective oral gestagen for humans (Borglin 1962)* with a powerful ovulation inhibiting effect (Ferin, 1962 /I/ and Kopera *et al*. 1963)

The drug Lyndiol® has recently been introduced on the Swedish market each tablet contains 5 mg lynestrenol + 150 γ mestranol (17 α-ethinyl 3-methoxy 1 3 5 (10)-oestratrien 17-ol) which is an orally active oestrogen Kopera *et al* (1963) have collated *a number of different tests of the anovulatory effect* of Lyndiol and lynestrenol on humans in cyclic oral medication The series comprised in all 2061 women with a total of 9496 cycles no pregnancy occurred. Pregnanediol determinations were performed on 88 women and showed consistently low values indicating anovulation. Laparotomy was performed in three cases in none of these was a recent corpus luteum found

It has recently been maintained that Lyndiol® has a reliable anovulatory effect on humans even at half dosage *i.e* 2.5 mg lynestrenol + 75 γ mestranol (Kopera and Ijzerman 1964) the present paper deals with a test of the latter preparation

The drug has been made available to us by courtesy of N V Organon, the Netherlands. It is to be introduced on the Swedish market by AB Pharmacia under the name of Lyndiol mite (known in other countries as Lyndiol 2 5)

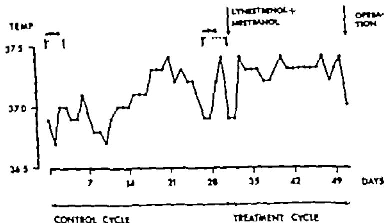


Figure Basal temperature chart in conjunction with administration of 2.5 mg lynestrenol and 75 γ mestranol. The characteristic rise at the commencement of treatment with the hormone combination shows that the preparation has a thermogenic effect.

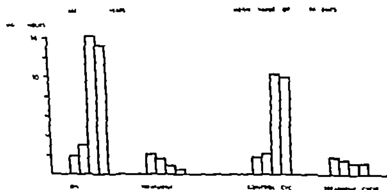


Figure Pregnanediol excretion in urine during treatment with 5 mg lynestrenol and 75 mestranol. The figure shows one typical case and the mean values for ten patients. The low pregnanediol excretion during the latter part of the treatment cycle constitutes strong evidence of inhibited ovulation.

routine analyses at the hospital s central laboratory GOT and GPT were determined by means of reagents on the basis of the DPNH method (Karmen 1955) The normal readings for both GOT and GPT were regarded as between 8 and 40 units.

All ovarian tissue was cut sagitally into blocks 3–4 mm thick. Corpora lutea above the size of a pea were measured in three diameters. In treated cases all blocks were embedded in paraffin, in control cases representative blocks only were embedded. All embedded blocks were sectioned at three levels and at each level three sections were taken These were stained with Masson s tri-chrome by the method of Ladewig, with periodic acid Schiff technique (PAS) by the method of MacManus and that of van Gieson The age of the corpus luteum was estimated by studying vascularisation amount of stroma and degenerative changes in granulosa lutein cells (Meyer 1911 1932 Papanicolaou *et al* 1948 and Novak and Woodruff 1962)

Results

Effect on Basal Temperature

In nine of the patients the basal temperature chart during the control cycle followed the normal ovulatory pattern with a distinct rise at the time of ovulation In one patient the chart was atypical but probably diphasic. However in this patient, the endometrium showed a secretory pattern on the 25th day of the control cycle During treatment with lynestrenol mestranol a rise in temperature was noted when the preparation was first given indicating that lynestrenol has a thermogenic effect. Fig 1 shows the basal temperature chart during the control and treatment cycles from one of our cases The basal temperature patterns of the other patients all showed the same characteristic rise at the commencement of hormonal treatment.

Effect on Excretion of Pregnanediol in Urine

In all the patients a marked increase in the excretion of pregnanediol in the urine was observed during the latter half of the



Fig 3a



Fig 3b



Fig 4

control cycle indicating that ovulation had occurred and a corpus luteum had been formed. On treatment with lynestrenol-mestranol, pregnanediol excretion remained virtually unchanged throughout the cycle an indication that ovulation had not taken place. Fig. 2 shows pregnanediol excretion during both cycles in one typical case and the mean values of pregnanediol excretion for the series as a whole.

Effect on Arborisation of Cervical Secretion

Arborisation of the cervical secretion was studied on the 8th and 21st days of both the control and treatment cycles. The results of these studies however were uncharacteristic and no conclusions could be drawn from them. While treatment was in progress the crystals typical of the proliferative phase were found in some cases while in other cases no such crystals could be detected.

Effect on Endometrium

Histological examination of specimens of endometrium taken by curettage from all patients on the 25th day of the control cycle showed normal secretory phase endometrium. Total hysterectomy was performed on the 25th day of the treatment cycle Histological examination of the endometrium in these preparations revealed in all cases the now familiar changes which occur after therapy with progestagens with or without oestrogen additives (Everse and Staland 1963) The mucosa was thin with few glands. The epithelium of the glandular ducts appeared for the most part in active although occasional indications of secretory phenomena were found. The surface parts of the stroma were oedematous. Areas showing clusters of marked decidual transformation of stromal cells were found in eight cases. Such areas often formed small polypoid indentations in the cavity

Figs. 3 and 4 show uterine mucosa from two cases Fig 3 a illustrates mucosa from the control cycle, and Figs 3 b and 4 from the treatment cycle.

A clear picture of adenomyosis of the uterus was found in three out of the ten cases.



Fig 3a



Fig 3b



Fig 4

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Table I shows the results of hepatic function tests during the control cycle (I) and during treatment with lynestrenol-mestranol (II). Abnormal values are shown in boxes.

Patient	Liver Function Tests										One Year after Treatment	
	Bilirubin		Alk. Phosphatase		Thymol Turbidity		GOT		GPT		GOT	GPT
	I	II	I	II	I	II	I	II	I	II		
		0 3				0 01				8		
	4	0 2	3		03	0 02	17		—	16	—	—
		0.2		4		0		5				
	4	4	5	3	0	0	33	23	—	30	—	—
		5		4		05		3		25		
3	0 7	4		3	04	3	6	26	36	38	—	—
		3				04		3		30		
4	4		4		02	03	5	26	48	90	20	19
				4		3		3		30		
5		0 2	3	3	13	17	23	60	26	52	27	7
		2		3				30		30		
6	3	2	4	3	02	0	23	42	36	130	40	5
				4		0.07		33		45		
7	0		4	3	09	05	25	28	23	61	32	57
		4		3		17		3		22		
8		3	1		—	09	6	7		22	—	—
		7		3		0		3		8		
9		0	3	3	01	02	5	3		5	—	—
				3				23		26		
	4	6	3		0 04	02	25	2	5	52	4	17

I = Control cycle
II = Treatment cycle

cycle. A further rise in these readings occurred during the treatment cycle.

GOT exceeded the normal limit (40 units) in two patients during the administration of the preparation. Comparison of the readings for the control and treatment cycles shows that there is a rise in GOT.

GPT gave higher readings in the second cycle in five patients, two of whom also showed a rise in GOT. One of these patients also had a slightly enhanced GPT reading during the control cycle.

Effect on Ovarial Tissue

No fresh corpus luteum was detectable macroscopically in the ovarian tissue examined from the ten cases in the treated group. When corpora lutea were found they invariably measured less than 7 mm in three diameters except in one case where the measurements were 10×6×6 mm Each of the four control cases displayed a fresh corpus luteum measuring approximately 14 mm in three diameters. Nor could the presence of any fresh corpus luteum corresponding to the current stage of the menstrual cycle be demonstrated histologically in the treated cases Since the patients were operated on the 25th day of the cycle in each case a fresh corpus luteum in a state of full maturity or in the earliest stages of regression would be expected. On the contrary all the corpora lutea found showed marked regression with pycnosis or disintegration of the nuclei in granulosa-lutein cells and often vacuolised cytoplasm containing coarse PAS-positive drops or hyalinisation in varying degrees.

In all cases a varying number of follicles in a late stage of maturity was found in the remaining ovarian tissue In no case did these follicles deviate from the normal size In one of the resected ovaries a prominent, possibly hyperplastic theca interna was observed in a few atretic follicles. In another case which displayed numerous groups of hilus cells adjacent to the rete ovarii vacuolisation was seen in several of these cells These phenomena could not be demonstrated in the other cases No other consistent changes in the ovarian tissue were observable

Effect on Hepatic Function

(see Table I)

Determinations of serum transaminases (GOT GPT) alkaline phosphatases serum bilirubin and thymol turbidity were performed once during the control cycle and on the 8th and 24th days of the treatment cycle Serum bilirubin and alkaline phosphatases showed quite normal readings in both cycles Two patients displayed a slight rise in thymol turbidity (0 13 for patient No 5 and 0 11 for patient No 8) during the control

macroscopic and microscopic examination of ovarian tissue showed that in no case had a corpus luteum been formed.

Lynestrenol has a thermogenic effect, so that records of basal temperature are of no help as a check on the inhibition of ovulation. A rise in basal temperature occurs immediately upon commencement of medication and not at the time of ovulation.

The absence of any increase in pregnanediol secretion in the urine constitutes very strong evidence that no ovulation has taken place but may not be entirely conclusive If the administered steroids affect the normal progesterone metabolism, it is conceivable that excretion of pregnanediol might fail to rise even if ovulation has taken place However there is no proof of any such influence on progesterone metabolism and the low rate of pregnanediol excretion in the latter part of the treatment cycles must be interpreted as evidence of inhibited ovulation.

The endometrium undergoes characteristic changes in conjunction with the administration of 2.5 mg lynestrenol and 75 γ mestranol. These changes are dependent on the direct effect of the administered hormonal combination on the mucosa, and do not constitute evidence of inhibited ovulation.

A study of the arborization of the cervical secretion does not give rise to any conclusions concerning the anovulatory effect of the preparation. During administration of the steroids, crystals typical of the normal proliferative phase can be observed in some cases, but not in others. The explanation of the inconsistent results may be that oestrogens form part of the hormonal combination and that for this reason typical crystals may be seen at any time during the course of treatment.

In this study the absence of corpus luteum in conjunction with the administration of 2 5 mg lynestrenol and 75 γ mestranol was confirmed in all cases by inspection of ovaries on laparotomy and subsequent microscopic examination of serial sections of ovarian tissue No inhibition of the development of the follicles was detectable in these cases In contrast to the reported findings of Rock, Garcia and Pincus (1957) and Ferin (1962) on treatment with gestagens alone. On these grounds, as well as the strong evidence of inhibited ovulation constituted by the failure of pregnanediol excretion to rise, we consider that the administered

Thus with GPT too a rise as compared with the control cycle was present. In two cases (patients Nos 5 and 6) the rise in GPT was considerable the figures being 153 and 130 units respectively The ratio GPT/GOT was >1 in the two patients showing a rise in the readings for both transaminases.

About one year after the investigation fresh blood samples were taken from those patients who had shown abnormal readings during the treatment cycle and the samples were analysed for GOT and GPT On this occasion there was a slight rise of GPT—57 units—in only one case (No 7) Transaminase readings were quite normal in the other patients who had shown high readings during the treatment cycle

Side Effects

Those side effects to which special attention was paid in this investigation were the occurrence of break through bleeding during the treatment cycle and subjective discomfort in the form of nausea In three cases bleeding occurred during the latter part of the treatment cycle and this was taken to be break through bleeding, though it should be recalled that every woman had a myomatous uterus None of our patients complained of nausea One patient stated that she had noticed an increasing feeling of tightness in the breasts during the treatment cycle but said that this was not of a painful nature No other side effects were recorded in connection with the administration of the steroids.

Discussion

The effect of a combination of 2 5 mg lynestrenol and 75 γ mestranol was studied by means of basal temperature pregnanediol excretion in urine and histological examination of ovarian tissue and endometrium. It has been claimed (Rock *et al.* 1957 and Matsumoto *et al.* 1960) that laparotomy with inspection and histological examination of ovarian tissue for the purpose of determining whether a fresh corpus luteum is present is the only absolutely reliable method of deciding whether or not ovulation has taken place In our series consisting of ten women

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hormonal combination is an effective anovulatory agent. The clinical experience referred to in the introduction concerning the testing of this steroid combination which was used as the only method of contraception by 854 women during a total of 4159 cycles without any pregnancy occurring, also supports this view

The subjective side effects of the preparation appear to be insignificant. A remarkable feature however was the incidence of rises in transaminases, although these rises were invariably moderate Whether they indicate a disturbance of hepatic function or not is very difficult to judge and further study of this phenomenon is needed before any conclusions can safely be drawn. There is no indication in the literature that the drug might have any deleterious effect on the functioning of the liver in women of fertile age (Swaab 1964) In postmenopausal women, however marked rises in GOT and GPT are observed after treatment with Lyndiol (Elsalo Järvinen and Luukkainen, 1964) Our experience suggests that a previous history of hepatic malfunction should contraindicate treatment with lynestrenol-mestranol.

SUMMARY

The inhibitory effect on ovulation of 2 5 mg lynestrenol combined with 75 γ mestranol was studied in ten women. The following features were studied in one control cycle and one treatment cycle basal temperature excretion of pregnanediol in urine arborisation of the cervical secretion careful inspection of the ovaries at laparotomy and histological examination of ovarian tissue and endometrium.

The preparation has a thermogenic effect. The excretion of pregnanediol does not rise during the latter phase of the treatment cycle indicating inhibition of ovulation At laparotomy and on histological examination of ovarian tissue no fresh corpora lutea could be found. In our opinion this proves that ovulation is effectively inhibited by the preparation

Side effects are low but we found in five of the patients a small rise of the serum transaminases GOT and/or GPT during treatment.

Frisk, M., Widholm, O. and Hortling, H. Acta obst. et gynec. scandinav. 44. [illegible] 1965
From the Outpatient Clinic for Teenagers, Samfundet Folkhälsan, Helsingfors

DYSMENORRHŒA—PSYCHE AND SOMA IN TEENAGERS

BY

M. FRISK, O. WIDHOLM, and H. HORTLING

Slight discomfort in connection with menstruation is a common occurrence but when pain of a more serious nature occurs, perhaps causing absence from work or school, the term dysmenorrhœa is warranted. This condition is frequently encountered. Gallhager (1960) reported a rate of 30 per cent among young girls, the symptoms being severe in 10 per cent of cases. In a series of young girls Gray (1960) noted a frequency of 27.5 per cent. Weiss and English (1957) studied a group of student nurses in which dysmenorrhœa was observed in 52 per cent. No relevant pathology was detected. The development of the condition was thought to be due to a combination of physiological weakness and a stress factor. In keeping with this, Gray (1960) reported a high incidence of uterine hypoplasia in cases of dysmenorrhœa. Widholm *et al.* in a series of young patients at the Outpatient Clinic for Teenagers observed dysmenorrhœa in 53 per cent, and in 46 per cent of patients without hyperœstrogenism. In a normal control series without hyper-œstrogenism the frequency was 17 per cent.

In addition to various gynæcological causes of dysmenorrhœa, the psychological background has been emphasized. Rogers (1964) thinks that in some cases the individual pain threshold may be lowered and the personal experience of physiological discomfort exaggerated. But according to Fluhmann (1958) among others, more basic psychogenic factors and mechanisms

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Table I. *The Causes for Seeking Medical Aid*

Gynaecological symptoms	56 per cent
Other somatic symptoms	58
Psychic symptoms	8
Impaired adaptation in the home	16
Impaired adaptation at school	4

Table II. *Gynaecological Symptoms and Findings*

Leucorrhoea	77 per cent
Premenstrual tension	30
Irregular menstruation	26
Hyperoestrogenous reaction	21
Relative hyperoestrogenous reaction	14
Hyperoestrogenous effect (total)	35

Table III. *Other Somatic Symptoms and Findings*

General vegetative symptoms	42 per cent
Fatigue	30
Headache	33
Nausea	
Functional abdominal pain	8
Obesity	25

Table IV. *Psychic Symptoms and Findings*

Insomnia	42 per cent
Impaired concentration	33
Emotional instability	28
Depression	28
Anxiety	26
Conflicts with the parents	9
Restlessness	4
Hysteroid reactions	

Table V. *School Problems*

Considerably impaired results	39 per cent
Aversion to school	9
Truancy	9
School tension	16
Problems connected with adaptation to gymnastics	7

Table VI. *Special Interests and Activities*

"Going steady" with boys	23 per cent
Member of juvenile church club	

must be taken into account in many cases in particular if the dysmenorrhoea occurs soon after menarche during the period of anovulatory cycles (Fluhmann, 1958 and Gray 1960) In connection with mental stress it has been observed that other gynaecological disturbances also tend to increase An increased frequency of hyper-oestrogenous effect in vaginal smears was detected by Frisk *et al.* (1964) in teenagers under mental stress, and Weiss and English (1957) reported an increased incidence of leucorrhoea in association with unconscious sexual ideas.

Material

In a series of 116 menstruating secondary-school pupils who visited the Outpatient Clinic for Teenagers on account of various symptoms 57 (49 per cent) had painful menstruation. When making appointments 56 per cent said that they had gynaecological symptoms. Equally often (58 per cent) the girls mentioned other somatic symptoms such as headache and fatigue Among the causes for seeking medical aid it is noteworthy that maladjustment and impaired adaptation were evident in 16 per cent of cases and subjective psychic symptoms in 18 per cent (Table I)

The duration of problems or symptoms was 2–3 years or more in two-thirds of the patients The girls were in their upper teens, the majority being 16–17 years old. The mean age of the series was 16.2 years, and there were disproportionate numbers of first and youngest children of families

Results

Dysmenorrhoea was observed in 49 per cent of cases. Affected patients frequently had both other physical and mental symptoms.

I *Gynaecological symptoms* There was a high incidence of leucorrhoea and of premenstrual tension. Hyperoestrogenesis was relatively common as judged by the appearances of vaginal smears. The vaginal cell reaction was considered high if more than 70 per cent of cells were acidophilic and karyopycnotic and without

In spite of the limited number of cases percentages are used in order to facilitate comparison.

Table VII. Gynaecological Findings

Hypoplastic uterus	28 per cent

Table VIII. *Somatic Findings*

Precocious development	25 per cent
Marked asthenia	18
Cerebral dysfunction	12
Obesity	5

Table IX. Family Background

Broken home	2 per cent
Father or mother physically ill	15
Father an alcoholic	9
Marital conflicts involving unfaithfulness	23
Mother attitude to child	
insecure	21
lacking in confidence	12
indifferent	24

Table X. *Psychic Mechanisms*

Overconformity	23 per cent
Inhibited aggressiveness	14
Sexual anxiety	12
Insecurity	26
Need for affection	
Problems regarding identity	26

here was a relatively high proportion of asthenic individuals nd a small group of girls with established dysfunction of the central nervous system. The large number of obese girls was noteworthy Otherwise these patients appeared to be in relatively good physical health (Table VIII)

III. *Psychic findings* Clarification of the psychological situation must take account of the *family background*. In many cases this deviated from the normal. There was either a broken home or the parents were ill, the father was an alcoholic, or there were marital conflicts. In addition, it was noteworthy that the attitude of the mother as characterized in part by insecurity in part by lack of confidence in the child and sometimes by indifference. The lack of confidence and the insecurity were fre-

progesterone effect. Menstrual irregularity was a relatively uncommon finding (Table II)

II *Other somatic symptoms* Various general symptoms, such as vegetative symptoms fatigue headache and functional abdominal pain were strikingly common (Table III)

III *Psychic symptoms* Various psychic symptoms particularly insomnia poor concentration emotional instability and anxiety were very frequently observed. Some girls displayed depressive reactions others had a tendency toward aggressiveness with resulting impaired adaptation and conflicts or they showed a regressive hysteroid type of reaction (Table IV)

The disturbed mental balance of the girls was also reflected in their school work. Various school problems occurred particularly in the form of a sudden impairment of school results frequently leading to failure to move up in class Sometimes the adjustment to school and school discipline was impaired, in other cases there was a tendency toward tension and anxiety in connection with school work or the adjustment to school gymnastics constituted a problem (Table V)

Certain features in regard to interests and activities deserve to be mentioned One-fifth of the girls were going steady with boys and one-tenth were members of juvenile church clubs (Table VI)

Clinical findings and observations of probable ætiological significance in regard to dysmennorhœa

I *Gynæcological findings.* A strikingly large number of the girls exhibited gynæcologically underdevelopment and hypoplasia of the uterus Pathological findings were otherwise few being limited to one case of endometriosis one follicular cyst and one case of suspected salpingo-oophoritis. The high frequency (35 per cent of cases) of hyperœstrogenism is noteworthy (Table VII)

II *Other somatic findings* It seems to be of significance that the series includes a group of precocious individuals with advanced bone age early menarche (mean age 12 6 years) and early development of the secondary sex characters. In addition,

toward school gymnastics associated with a fear of being seen through an escape from sexuality by resorting to juvenile church clubs, or regressive reactions (Table X)

Dicussion

The present study which was performed mainly on female secondary-school pupils in their later teens reveals a rise in the frequency of dysmenorrhoea in connection with increased and conflict-laden psychic tension. Mostly however dysmenorrhoea was not the only symptom. It was found to be associated with an increased frequency of other gynaecological somatic, and psychic symptoms.

A constitutional weakness in the form of uterine hypoplasia observed in one-third of the cases was a somatic factor of importance. In some instances this developmental disturbance seemed to be the primary cause of the symptoms, but often it may be assumed that various other factors were also involved e g stress and a punctum minoris resistentiae Furthermore in evaluating the condition and the total psychophysical reactions of these girls the high frequecy of asthenia the disturbed functional vegetative balance and the tendency toward obesity should be borne in mind.

As a rule psychic tension was the main feature. This was usually associated with anxiety or aggression against the background of a conflict situation, with overconformity deviating outer or inner claims, insecurity and problems regarding identity It was in keeping with the stress situation of these individuals that abundant psychic symptoms occurred, although they did not constitute the cause for seeking medical aid. The disturbed inner balance was reflected in poor adaptation and impaired capacity for work, with school problems and lowered marks resulting. As a rule, the school problems were the result of the crisis and not its cause. Frequently the conflict had a special character owing to experiences of a sexual nature and owing to the occurrence of sexual problems a year or two after the menarche. This seems to be of greater significance than the occurrence of ovulatory cycles in the development of painful menstruation. In a

quently associated with sexual anxiety The indifference and the disturbed emotional relationship seemed to imply a rejection of the child due to various personal conflicts and problems (Table IX)

Psychic mechanisms. In some cases the mental tension was found to be connected with social insecurity and a need for over conformity associated with a broken home or illness of the parents The overconformity the need for desisting from personal claims excessive emotional attachment and, inability to establish adult identity in these instances created a conflict situation.

In connection with marital conflicts and paternal alcoholism another conflict situation was encountered, in which inhibited aggressiveness and tension were fundamental constituents.

In addition sexual problems occupied a central position. These problems were sometimes connected with premature development and its secondary consequences which neither the child herself nor her parents were able to cope with and master Occasionally the attitude of the parents in these cases seemed to be characterized by overprotectiveness and a fear that sexual problems might arise In some cases the mother's primary over protective attitude seemed to be due to her personal sexual difficulties or marital problems. A conflict situation in the home with marital unfaithfulness etc. was seen to elicit a sexually centered anxiety Sexual problems also resulted from a strong need for affection due to emotional deprivation which was satisfied by increased association with boys. This intimacy activated sexual feelings and led to sexual contacts in 16 per cent of cases.

Owing to an early disturbance of the ego development, or owing to a crisis in connection with puberty the struggle for identity was apparently rendered more difficult. The development of these girls seemed to be obstructed because the integration of sexuality the recognition of the feminine sexual role constituted a problem for which disturbed identification was responsible In some cases there was a fear of growing up due to traumatizing experiences of premature and threatening sexuality Essential features in this struggle for sexual identity were among other things unwillingness to accept their own body which in some cases obviously led to excessive eating, a rejecting attitude

ous other factors were also involved, e g stress and a *punctum minoris resistentiae* Pathological findings were otherwise few The series includes, however a group of precocious individuals Psychic tension was the main feature associated with anxiety or aggression against the background of a conflict situation with overconformity deviating outer or inner claims insecurity and problems regarding identity Frequently the conflict had a special character owing to experiences of a sexual nature.

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series from the Outpatient Clinic for Teenagers, Widholm (1964) has shown that dysmenorrhoea also occurs in connection with anovulatory cycles. In the present study different psychic conflict mechanisms were recognizable including traumatizing, anxiety laden fantasies regarding marriage family life and sexuality as a love symbol. In addition the establishment of sexual identity seemed to be barred by mechanisms preventing the child from growing up from finding the appropriate sexual role. A deviating attitude to sexual matters, in particular a projection of the mother's anxiety seemed to be reflected in these conflicts. In many cases it may be assumed that the girl's physical prematurity had elicited anxiety in the parents and thus placed the experience of sexuality in focus at a time when the individual otherwise was not mature enough to be confronted with this new personality trait. On the other hand, emotional deprivation due to lack of contact with the parents obviously predisposed to a need for affection and to sexual problems, occasionally leading to real guilt-laden sexual contacts. In the present cases of dysmenorrhoea the sexual feeling was very often a danger to the individual a threat against her self-esteem and security.

SUMMARY

In a series of 116 post-menarchal secondary-school pupils the majority being 16–17 years old who visited the Folkhälsan Out patient Clinic for Teenagers 49 per cent had dysmenorrhoea. Widholm *et al.* (1964) found that dysmenorrhoea occurred in 25 per cent of a normal control series of girls. In the present series there was also a high incidence of leucorrhoea premenstrual tension and hyper-oestrogenism as judged by vaginal smears. Various general symptoms such as vegetative lability fatigue headache functional abdominal pain, psychic anxiety emotional instability insomnia and poor concentration were observed. A disturbed mental balance was also reflected in school work. Twenty-eight per cent of the girls exhibited gynaecologically demonstrable underdevelopment and uterine hypoplasia. In some instances this developmental disturbance seemed to be the primary cause of the dysmenorrhoea but often it seemed that vari-

Table I. *Results of Cervical and Vaginal Cytology in a 16-Month Period*

	Number	%
With tumour cells	31	3
With suspicious cells	46	1.9
With atypical cells	43	1.8
Without abnormal cells	230	95
Total	2421	00

slide, where it deposited a film. The specimen was immediately fixed in equal parts of ether and 96 % alcohol, and the preparations were stained with EA 50 by the method of Papanicolaou.

The examination of all the smears was carried out by pathologists.

Abnormal cells were classified as tumour cells suspicious cells or atypical cells.

Atypical cells are encountered particularly in the presence of inflammatory conditions and cannot generally be interpreted as signs of malignancy

In cases where the material was not suitable or "inadequate the sampling procedure was repeated.

A histological diagnosis of carcinoma was made only in the event of invasive growth. The term precancerous lesion was used in cases showing exclusively intraepithelial changes.

The series comprises 2421 specimens. The results are listed in Table I.

Abnormal cells were demonstrated in 120 specimens. In 3.2 per cent of the specimens, cytology gave rise to a suspicion of malignancy while in 1.8 per cent there were atypical cells which did not suggest malignancy

Comparison with other gynaecological series is potentially inaccurate as the selection of cases may vary partly because of varying proportions of in- and out-patients, and partly because of a varying number of women without gynaecological symptoms may be included. In addition, the differentiation between the various types of abnormal cells varies. Some authors like us state

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VALUE OF ROUTINE CERVICAL AND VAGINAL CYTOLOGY IN GYNÆCOLOGICAL PATIENTS IN ORDER TO DETECT TUMOUR CELLS

BY

E. BREDAHL AND H. LEFÈVRE

Investigation of cervical and/or vaginal smears for tumour cells is recognized as a valuable aid in diagnosing cervical carcinoma and precancerous conditions among women in large groups of the population

On the other hand opinions are divided concerning the value of this diagnostic method when used as a routine procedure in a gynæcological department.

A decisive factor is whether this method results in the diagnosis of cervical carcinoma and precancerous conditions which would not have been disclosed by ordinary diagnostic methods.

Routine cytological study of vaginal smears from all gynæcological patients would so increase the burden of work in the pathology departments that it seems justified to investigate whether this increased work is in a reasonable proportion to the benefit of such studies

In an effort to elucidate this aspect, the majority of gynæcological patients admitted to the Copenhagen County Hospital, Glostrup during a period of 16 months were submitted to a routine cytological study of cervical and vaginal smears.

The investigation was carried out in all patients except in those with profuse vaginal bleeding

Samples were removed from the cervix and from the vaginal pool with a cotton tipped stick which was then rolled over the

Table III. Cytological Findings in Uterine Malignancies

	Total	Positive Cytological Findings	Negative Cytological Findings
Cervical carcinoma	27	24	(3)
Precancerous lesions of the cervix	22	9	(3)
Endometrial carcinoma	9	5	(4)
Total	58	48	(10)

vaginalis in some cases only after a positive cytological report had been received.

Lastly there is a group of 22 patients in whom these procedures were not felt to be indicated. Three cases with suspicious cells were regarded as "false positive" results (*cf.* Table IV) and in 19 cases atypical cells were found.

The results of the histological studies are shown in Table II in which the false positive results are listed in brackets.

The table shows that malignant and pre-malignant changes were demonstrated in 49 out of the 80 patients investigated, on the portio vaginalis in 44 cases, and in the corpus in 5.

Among the 24 patients with carcinoma of the cervix rather more than half showed tumour cells and less than half showed suspicious cells in their smears. Among the 20 patients with precancerous lesions of the cervix about half showed tumour cells. Among the 20 patients with atypical cells, there was one with endometrial carcinoma and there were 3 with precancerous lesions. Among the 60 patients with tumour cells and suspicious cells, 24 had cervical carcinoma, 18 precancerous lesions of the cervix and 3 endometrial carcinoma. In 5 cases the specimen was inadequate for a histological diagnosis. The 13 cases with normal findings or benign changes must be designated as "false positive". To this group we have also assigned the two cases in which the specimen was inadequate. The specimens from these 15 patients have been reviewed and the diagnosis revised (*cf.* Table IV).

During the 16 months of the study 58 cases of uterine malignancy were diagnosed histologically. The cytological findings in

Table II. *Correlation of Cytological Diagnosis with Histological Diagnosis*

Histological Diagnosis	Cytological Diagnosis			
	tumour cells	susp. cells	atyp. cells	total
Benign changes of the portio and cervix	(1)	(10)	10	1
Precancerous lesions of the portio	11	7	2	20
Cervical carcinoma	13	11	0	24
Benign endometrial changes	0	(2)	3	5
Precancerous endometrial lesions	0	0	1	1
Endometrial carcinoma	2	1	1	4
Inadequate material	0	2	3	5
Total	27	33	20	80

the percentage of specimens showing abnormal cells (Burge and Carlsson 1956) while others state the percentage of patients showing abnormal cells (Hartford, 1955 Messelt, 1955 Scheffy and Rakoff 1948)

Out of the 120 cases in which abnormal cells were demonstrated 89 per cent showed these abnormal cells in the cervical as well as vaginal smears while 8 per cent had abnormal cells only in the cervical and 3 per cent only in the vaginal smears. This shows that the cervical smear is of the utmost importance in demonstrating abnormal cells. This is in keeping with the results of others (Cuyler *et al* 1951 1952 Messelt, 1955 Slate *et al.* 1953 and Song *et al* 1959) As far as endometrial carcinoma is concerned, however several authors have reported that abnormal cells are more commonly found in the vaginal than in the cervical smear

The 120 specimens showing abnormal cells are from 102 patients about 66 per cent of whom were in the age group 30–49 years and about 10 per cent under 30 years of age

In the 63 of the 102 patients tumour cells and/or suspicious cells were demonstrated while in the remaining 39 only atypical cells were seen

The majority of the patients in whom abnormal cells were found had fractionated curettage and/or biopsy from the portio

Table IV *Revision of "False Positive" and "False Negative" Cases*

Revision of "false positive" cases	
Tumour cells altered to suspicious cells	1 case
Suspicious cells altered to atypical cells or no tumour cells	cases
Unsuitable material	1 case
No alteration	3 cases
Total	7 cases
Revision of "false negative" cases	
No tumour cells altered to suspicious cells	case
Unsuitable material	8 cases
Total	9 cases

On these grounds it may be argued that the 18 "false positives" in the present series may not have been "false positives" at all, as no conization was performed.

The cardinal question must be whether the routine investigation of the cervical and vaginal secretions was of importance in diagnosing malignant and pre-malignant uterine diseases in a gynaecological department in which there are extremely wide indications for punch biopsy and fractionated curettage

A review of the records has shown that the method was of decisive importance in diagnosing precancerous conditions in 4 cases in which the gynaecological examination did not indicate the need for a biopsy from the cervix.

In another 4 cases (one with carcinoma and 3 with precancerous lesions of the cervix) in which a biopsy was not primarily removed from the cervix, the cytological findings were positive A concurrent fractionated curettage revealed small flakes of undifferentiated squamous epithelium from the cervix.

An assessment of the method must also include a revision of the 18 "false positive" and the 10 "false negative" cases. There was a possibility of reviewing 26 out of the 28 specimens. The results are shown in Table IV

It is evident that in 3 cases the revision detracted from the cytological diagnosis while in one case suspicous cells had been overlooked. In 9 out of the 26 cases the specimens ought to have

these cases are recorded in Table III (false negatives in brackets)

In other words in this series of 2421 specimens cervical carcinoma was demonstrated in 24 patients precancerous lesions of the cervix in 19 and endometrial carcinoma in 5 patients while there was a failure to make the diagnosis by cytological examination in 10 cases The specimens from these patients were reviewed and the diagnosis revised (*cf* Table IV)

Histological examination also carries a certain inaccuracy as is shown in other series This is partly due to the varying case materials—as already mentioned—and partly because the criteria for precancerous lesions may vary from series to series.

Major series have been *published* by Andersson 1959 Boddington *et al.* 1960, Cuyler *et al.*, 1951 1952 Lombard *et al.* 1952 Markley *et al* 1955, and Slate *et al.* 1953

The question now arises Is cytological study superfluous when there is occasion to obtain a biopsy and/or carry out fractionated curettage?

The indication for obtaining a biopsy must be based on the gross appearance of the portio vaginalis.

In the present series the cervix was of normal appearance in 45 of the patients with abnormal cells. Histological study of a biopsy revealed precancerous lesions in 5 patients Moreover among 22 patients with abnormal cells and a benign-looking erosion 3 *had* carcinoma and 10 precancerous *lesions* of the cervix Thus out of the total of 102 patients with abnormal cells, 67 (about two-thirds) had a normal cervix or a benign looking erosion and in this group 3 cases of carcinoma and 15 of precancerous *lesions* of the cervix were *disclosed That a normal*-looking cervix does not exclude carcinoma or precancerous conditions has also been emphasized by among others Cuyler *et al* 1951 1952 Estrada *et al* 1959 and Nelson *et al* 1960

Furthermore several authors have pointed out that a negative punch biopsy does not rule out a malignant or pre-malignant condition (Cuyler *et al.* 1951 1952 Estrada *et al.* 1959 Hartford, 1955, and Sheffey and Rakoff 1948) Consequently *conization is recommended in the event of* positive cytological findings and a negative punch biopsy

As a general rule, cytological examinations are restricted to patients in the age range 25–55 years.

Unsuitable material is rejected and a new specimen is asked for

Any abnormal cytological finding is followed up by biopsy and curettage, and possibly further procedures after consultation in each individual case.

SUMMARY

During a period of 16 months cervical and vaginal smears from all gynaecological in-patients were submitted to cytological study This comprised 2421 specimens. 120 specimens (5 per cent) from 102 patients, revealed abnormal cells. In 8 per cent the abnormal cells were found only in the cervical smears in 3 per cent only in the vaginal smears. Cervical carcinoma was found in 24 patients precancerous conditions of the cervix in 20 patients and malignant endometrial changes in 5. There were 13 false positive cases in this group During the same period histological study revealed 27 cases of cervical carcinoma in 24 of which cytology had been positive and in 3 false negative 22 cases of precancerous lesions of the cervix in 19 of which cytology had been positive and in 3 false negative as well as 9 cases of endometrial carcinoma in 5 of which cytology had been positive and in 4 false negative Among the 102 patients with abnormal cells 45 had normal macroscopic appearances of the cervix. Five of these patients had precancerous lesions. Twenty two patients had a benign-looking erosion. Three of these patients had carcinoma of the cervix and 10 precancerous lesions. Revision of the false positives and "false negatives showed that the main cause of failure was poor-quality specimens which ought to have been rejected.

In 4 instances the study was of decisive importance in diagnosing precancerous conditions of the cervix. In another 4 cases, in which biopsy was not obtained primarily the method would have played a role in diagnosing one case of carcinoma and 3 cases of precancerous cervical lesions if the curettage had not included small flakes of undifferentiated squamous epithelium.

It is concluded that the ideal practice would be a cytological

been rejected. This shows that the demands on the quality of the specimens should be stricter In this respect, immediate fixation is presumably of the utmost importance

The fact that there seemed to be no reason to alter the cytological diagnosis in half the cases perhaps indicates that the criteria of the cytological signs of malignancy are too wide. However if the criteria are rendered stricter in order to reduce the number of false positives" the number of false negatives must be expected to increase as also pointed out by Graham 1954. The object is to diagnose all carcinomas and precancerous conditions and one must accordingly accept a certain number of false positives" although they do give rise to some unnecessary diagnostic procedures. However some of the false positive cases are due to inflammatory conditions which in any event would require treatment.

Conclusion

On the background of this study it must be concluded that cytological study is indispensable in a gynæcological department.

It is primarily of importance in disclosing precancerous conditions in patients in whom gynæcological examination has not given rise to a suspicion of such conditions.

Since a normal looking cervix and a negative punch biopsy from an erosion do not rule out precancerous conditions the ideal procedure would be a routine investigation of the cervical and vaginal secretion in all gynæcological patients.

It is possible by means of cytological investigation to pick out patients who require a more thorough investigation (fractionated curettage multiple punch biopsies from the portio conization)

Routine cytological examination of all gynæcological patients would require a larger staff particularly cytotechnicians trained to screen all the preparations but this is not practicable at present

Instead, the practical consequences of the present study in the gynæcological department and the pathological department have been as follows

As a rule no cytological examination is done in cases submitted to biopsy and fractionated curettage

Only cervical smears are studied.

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URETHROCYSTOGRAPHY — METHOD AND DIAGNOSTIC ASPECTS

BY

C.-E. UNNÉRUS, P. ESTOLA AND G. KROKFORS

Modern diagnostic aids and methods have considerably improved the chances of analyzing the different types of complaints arising from the lower urinary tract in women. As elsewhere the rule applies that thorough knowledge of the normal conditions is necessary for correct evaluation of the nature of pathological changes. Great difficulties have been encountered in this respect especially in the region of the fundus of the urinary bladder and the urethra. Opinions concerning both their anatomy and function have been conflicting for a long time.

The urinary bladder and the urethra are associated so closely with the anatomy of the other organs in the true pelvis that a gynaecological evaluation, for instance, is always required. Cystoscopy, cystometry and sphincterometry and roentgenologic cysto-urethrography may be mentioned as examples of the wide range of methods which are necessary for assessment of the state of the lower urinary tract.

In order to obtain a truly reliable view of the functional circumstances, cinematography should be used in the roentgenologic control as was done e.g. by Ardran et al. 1956 and Enhörning, 1960. However, only a few hospitals have the requisite apparatus and consequently it is necessary to resort to other methods of examination. In any case cinematography involves fairly high radiation doses, a point which is of particular importance in examining women of fertile age.

examination of all gynæcological patients, but as this is not practicable at present because of staff shortage the following consequences have been drawn from the study. Only cervical smears are examined. No cytological study is carried out in cases where a biopsy specimen from the cervix is obtained or fractionated curettage is performed. Only patients between 25 and 55 years of age are investigated cytologically and abnormal cytological findings are followed up by curettage and biopsy from the cervix.

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physiological conditions. It is not necessary with this method to use the chain and metal balls which have been employed by many other authors, *e.g.* Hodgkinson 1953, and Calatroni *et al.* 1962.

After the catheter has been withdrawn a thin metal clip is applied to the external urethral meatus, which is thus visualised on the roentgenogram. Anteroposterior and lateral exposures are made with the patient supine. A third roentgenogram, also a lateral view is then taken with the patient in a standing position, which is easily done on a tilting examination table.

For a micturating woman the sitting position is physiological. However the study of micturition in the sitting position hitherto has been difficult due to the lack of equipment suitable for X-ray examination of the bladder and the urethra. Consequently one of the authors (Unnérus) constructed a portable water closet of plexiglass, which has about the same radiation absorption as human soft tissues. With lateral roentgenograms however the density of the thighs is added to that of the pelvis which necessitates the use of hard X rays. In order to avoid over exposure of the soft parts *e.g.* the urethra, Unnérus constructed an aluminium wedge filter which is applied to the back of the fluorescent screen close to the cassette. This filter produces an even absorption of X rays and results in a balanced density throughout the roentgenograms.

Another lateral exposure is taken in the sitting position while the patient is straining. Exposures are then taken in quick succession during micturition. It has sometimes proved useful—especially for assessment of patients who have been operated upon—to take a further roentgenogram by the so-called double exposure technique. This means that the situation both on straining and voiding is visualised on the same picture. A final roentgenogram is made in the anteroposterior direction after urination in order to confirm whether the bladder has been emptied completely. The metal clip is removed after ensuring that the examination was successful.

Figs. 2–4 illustrate the results.

It is important that the urethra is visualised in its entirety in the roentgenograms during micturition and it is possible to screen



Fig. 1 A silver clip has been applied to the external meatus of the urethra. Note the good visualisation of the entire urethra and the even density of the whole picture—this has been achieved in spite of the varying density conditions by using a wedgeshaped aluminium filter

To be able to give an idea of the functional conditions in addition to the purely anatomic facts the present authors have evolved a roentgenologic examination method which has now been used for some 4 years on over 2000 patients. It is simple enough for use in practically any roentgenologic department with only slight additions to standard equipment.

Method

Sterile barium sulphate suspension is used as the contrast medium. 350–400 cc. of the suspension is introduced into the urinary bladder via a rubber catheter which is then withdrawn. This contrast medium does not irritate the bladder and also gives excellent contrast in the relatively thin urethra As it is not necessary to use an anesthetic agent, the examination is close to



Fig 4 State on micturition Note the angles between the urethra and urinary bladder



Fig 5 A protractor has been applied to facilitate visualization of the rotation of the bladder from the resting to the straining position.

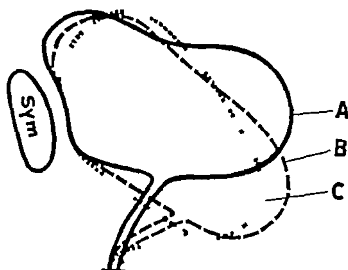


Fig. 2. This diagrammatic picture shows the state in the region of the neck of the urinary bladder A = rest, B = on straining and C = on micturition.



Fig. 3. State in the sitting position at rest.



Fig. 6 The distance between the urethra and the dorsal surface of the symphysis has been shortened operatively and a "pull" has begun to develop. The bladder neck has been dilated permanently causing incontinence.

to the symphysis and obturator foramen. The authors found that a line drawn between the lower margin of the obturator foramen and the lower margin of the symphysis (which anatomical parts are easily recognized on the roentgenogram) indicates a lower limit for a normal position of the bladder fundus. The authors feel that this facilitates the diagnosis which is otherwise often distorted by errors of projection or other incompatible data.

3 and 4 The anterior and posterior angles between the bladder and the urethra are of great diagnostic significance. If the anterior angle has become straightened out, the conditions for incontinence exist. This may happen e.g. if the tissue between the symphysis and the anterior wall of the bladder has become shortened as a result of post-operative cicatrisation. This reduces the mobility of the urethra and, with time causes some shrinking and pulling of the wall of the bladder and the urethra. A similar result may be produced by operative fixation of the bladder to tissues situated

the fundal part of the urinary bladder to a considerably extent. If the technique is good, the radiation dose can be kept small, a point that has exercised the attention of the authors throughout (Unnérus *et al*, 1963)

Earlier most of the examinations were done with patients in a standing position which the authors regard as less physiological.

Diagnostically important aspects

Much information on the anatomy and function is obtained by performing the examination in the supine and standing positions, and during both straining and voiding in the sitting position. There is no relation between the degree of descent of the pelvic floor and the urinary bladder on one hand and urinary incontinence on the other Prolapse may be the consequence of the strain and trauma of delivery or may result from a connective tissue weakness of the kind often encountered in elderly women. Hormonal factors obviously play an important role The cause of the incontinence is to be found in the variations in fixation and the tissue layers of the urethra Langreder 1956 and 1961 Youssef 1960, Low 1961 and 1964 Newman 1949 Enhörning, 1961 Järvinen *et al.* 1961 Lapides 1961 and many other authors have analysed the anatomic and functional background of these prolapse and incontinence phenomena and for the relationship between causes and effects reference is made to these comprehensive reports However special mention might be made of the observations by Enhörning (1961) regarding the difference in pressure The pressure in a normal urinary bladder at rest is 5–10 cm H_2O during urination in the sitting position 20 cm and over and during coughing and straining as high as 90 cm H_2O

The following facts should be considered in the roentgenologic evaluation

1 The distance between the anterior wall of the bladder and the symphysis. A lengthening here creates the prerequisite conditions for prolapse and incontinence while the shortening that is often seen after operations results in reduced urethral mobility which in certain cases may also result in incontinence suggesting that the operative method was incorrect.

2 The position of the fundus of the urinary bladder in relation



Fig 7 A functionally good result has been achieved with a suitable operative method despite considerable dilatation and prolapse of the urinary bladder

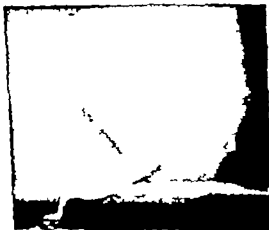


Fig 8 The line joining the lower margin of the symphysis and the lower margin of the obturator foramen makes good reference against which to observe the position of the urinary bladder—especially when the amount of contrast medium in the bladder is about 150 cc.

higher up In such a case the reason for the prolapse or incontinence has been incorrectly assessed and the reparative technique was unsuitable

The first to draw attention to the dorsal urethro-vesical angle which is about 100 degrees at rest and on straining in a normal continent woman were Jeffcoate and Roberts 1952 It becomes more obtuse on micturition over 110 degrees. The posterior angle is not influenced by the angle of the posterior floor of the position of the bladder neck.

5 Displacement of the urethra in the ventral or dorsal direction. This is often associated with cystocœle or uterine prolapse but may also be due to fixation by scar tissue following trauma or operation

6 The course of the urethra itself is of some importance. Clearly the mucous membrane of the urethra is also subject to *variations which are apparently of hormonal nature (cf* Johanson—Järvinen 1957) for instance the resistance in the urethra *may vary markedly as the result of œdema* of the soft tissues. This is important especially when examining women of fertile age when it is necessary to note the phase of the menstrual cycle at the time of the examination and perform subsequent examinations at the same phase

7 Observations concerning the rotation of the urinary bladder It is possible to measure the degree of rotation by examining the patient in the manner described above In some of the cases the present authors used for this purpose a protracter fixed against the cassette Normal rotation amounts to 70–90 degrees.

This observation is important when deciding whether operation is indicated and what surgical technique should be employed because it gives an idea of the status of the muscles.

In addition to these seven *important factors there are* naturally all the ordinary observations that can be made in the different *projections* of various stages of filling, dilatation of the urinary bladder the lumen of the urethra and morphologic changes mucosal relief etc

The rœntgenologic examination thus offers a multiplicity of observations which contribute as a whole to the analysis of the patient s complaint.



Fig 7 A functionally good result has been achieved with suitable operative method despite considerable dilatation and prolapse of the urinary bladder

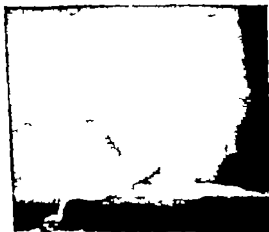


Fig 8 The line joining the lower margin of the symphysis and the lower margin of the obturator foramen makes good reference against which to observe the position of the urinary bladder—especially when the amount of contrast medium in the bladder is about 50 cc.

These examinations are invariably supplemented at both the university departments of obstetrics and gynaecology in Helsinki by ordinary gynaecological and cystometric examinations (Järvinen *et al.* 1961).

Discussion

The clinical criteria for the evaluation of incontinence in woman are satisfactory. The difficulty remains to decide the correct indications for operative intervention and to choose the most suitable surgical technique. As the complaints and the pathological status are often of diverse aetiology, a more comprehensive but consequently more time-consuming restoration of the status in the pelvic region should be performed more frequently, as was pointed out, e.g. by Turunen as long ago as 1938. Muellner (1959) reported the descent of the fundus of the bladder and its funnelshaped bulging to be complicating factors. The importance of the increased distance between the urethra and symphysis and the descent of the neck of the bladder was pointed out by Ball, 1950, Mikulicz-Radecki, 1931, and Ingelman-Sundberg, 1949. Hodgkinson (1953) stressed the significance of the finding that the bladder in its entirety is displaced in the vertical direction so that the internal meatus of the urethra lies at the lowest point. Both Youssef (1960) and Langreder (1956) stated that there is no real annular sphincter musculature in the urethra, but that the muscle fibres from the bladder pass without sharp demarcation to the urethra. The urethra, however, is normally fairly firmly fixed at the function of its distal and middle thirds and thus is visualised as an angle formation in normal roentgenograms taken in a resting position.

The operator must note, e.g., which anatomic factors counteract the prolapse of the fundus of the bladder and the region of the neck of the bladder. The most important of these factors are the lavator muscles and pelvic fascia, the fixation between the posterior wall of the bladder and cervix uteri and between the bladder and the parametria. If an operation is indicated, the most suitable method must be assessed: an anterior vaginoplasty or a retropubic urethroplasty or a combination of the two.

The consequences of different operative measures are beyond

the scope of this paper The departments of obstetrics and gynæcology in Helsinki use chiefly the methods of Marshall, 1949 Marchetti, 1949 Krantz 1951 and Ingelman-Sundberg, 1952 or the modifications that they developed themselves.

Figs. 6–7 illustrate operative results, some aspects of which can be discussed.

The authors wish to point out, that the urethro-vesical junction should normally be 2–3 cm from the dorsal surface of the symphysis.

The importance of control examinations after operation needs to be stressed. At first, and persisting for some time 2–4 months or so there is fairly considerable tissue œdema, which seems to be of functional significance in that continence may depend on it. But when the patient is followed up in 2–4 years new findings are made and scar formation is prominent. This is of importance, if a repeat operation is contemplated and may involve considerable technical difficulties. Repeated surgery is also physiologically bad for the patient.

The authors have arrived at the conclusion that a general examination of the pelvic organs should precede these fairly comprehensive operations more often than has been the practice previously Such examination also makes it possible to assess better the musculature and parenchymatous organs of the pelvic region as a whole For instance hysterography should always be performed concurrently and also roentgenologic visualisation of the position and function of the organs during defæcation. In particular the relations between the urethra and cervix and the vaginal walls, should be analysed more thoroughly This is easily done by simple means.

It is important that the digital examination is done with the patient in different positions and with her bladder filled.

The techniques for examinations and operation suggest that no method of universal applicability has been found so far and that many different methods are necessary for correct analysis and management of the varying circumstances. On the other hand, it is of the greatest importance to progress so far in diagnostic knowledge that it will always be possible to select a suitable method of operation.

SUMMARY

The authors have devised a method of urethrocystography which is relatively easy to perform in a normally equipped roentgenologic department—without cinematography It is important that micturition takes place in a sitting position and is preceded by straining efforts. Barium sulphate suspension was found to be an eminently suitable medium in over 2000 cases studied.

The authors analyse the most important diagnostic viewpoints to be considered. The indications for operation are also discussed. Some new diagnostic criteria are advanced among which the greatest importance is attached to the position of the neck of the urinary bladder in relation to the lower margin of the obturator foramen.

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THE TECHNIQUE OF PELVIC PHLEBOGRAPHY

BY

RICH. HAMMEN

In an attempt to diagnose various causes of pelvic pain in women for six years we have been examining the venous circulation of gynaecological patients by means of pelvic phlebography Various approaches for the introduction of contrast medium such as penetration through the pelvic bones the veins of the leg, and the vaginal portio of the uterus have all been tried and discarded. The technique finally adopted was injection into the uterine fundus as advocated by Topolanski Sierra (1957)

Technique

The patient is placed in the lithotomy position on the X-ray table The portio vaginalis is wiped with a sterile gauze swab and the os uteri cleaned with merbromin or iodine The uterus is grasped on either side of the os by a tenaculum

The cannula is introduced through the cervical canal and plunged two to four mm into the myometrium of the fundus. First hyaluronidase (penetrase) 500 units is injected by syringe followed by the contrast medium (urografin 76 %—20 ml) The injection is completed in less than 20–30 sec The first picture is taken immediately, followed by pictures two three four at intervals of 20 sec. The last and fifth picture is taken one minute later

Our initial experience with the technique and instruments described by Topolanski Sierra showed that the results, although sometimes good, were haphazard The reasons for failure were

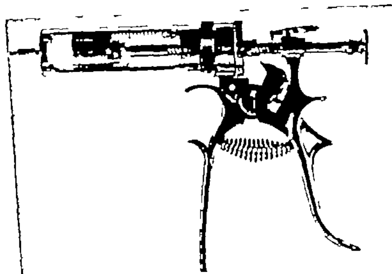


Fig. Revolver syringe for phlebography

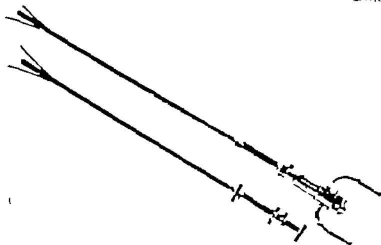


Fig 2 The double cannula —The upper model is for the double syringe (Fig 3)

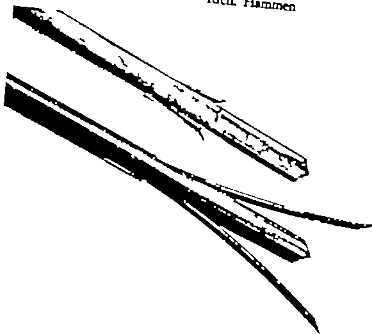


Fig 2 b Tip of the cannula.

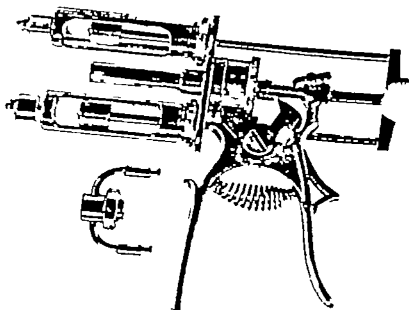


Fig. 3 Double syringe



Fig 4 Pelvic varices

1 Complete penetration of the uterine wall with injection into the peritoneal cavity especially where the uterus was bulky and soft, as in cases of pelvic congestion.
2 Injection other than in the mid-plane of the uterus results in asymmetric filling of the vessels.
3 Resistance of the tissues to injection sometimes makes examination impossible.

We therefore changed the method rather radically To overcome the pressure we replaced the instrument by a revolver syringe the handle of which was modified to obtain a higher pressure and to prevent the piston from being ejected by the back pressure (Fig 1)

To obtain symmetrical distribution of the contrast medium in *the pelvis we used two cannulæ one inserted into the myometrium* on each side of the midline of the uterus We endeavoured to eliminate complete penetration of the myometrium by binding the cannulæ together by fanning them out so that they enter the myometrium obliquely and by adjusting the length of the free tips of the cannulæ individually The two cannulæ are enclosed in a steel sheath during the introduction the circumference of which is equal to Hegar No 4 (Fig 2 a)

Our pictures using this technique have usually been quite good. However sometimes asymmetrical filling of the venous system occurs and this seems inevitable occasionally It may by chance, happen that one cannula goes into the lumen of a vein, while the other one is situated in dense myometrium which offers much resistance In these circumstances the contrast medium takes the line of least resistance into the venous system.

For this reason the apparatus has been further modified and we now use two separate systems two syringes and two cannulæ but both systems are directed by the same power (Fig 3) It ensures that the speed of injection is regulated by the area of greatest counterpressure and no contrast medium passes into the vein before the high pressure in the other system has been over come

The examination is made very easily We have seen no complications in approximately 200 cases except occasional moderate pain in the lower abdomen We therefore give Morphine-Scopolamine premedication

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FOLIC ACID AND REPRODUCTION[1]

BY

B. M. HIBBARD, ELIZABETH D. HIBBARD AND T. N. A. JEFFCOATE

The dramatic fall in the maternal and peri-natal mortality rates which occurred in most Western societies during the first half of this century is attributable mainly to better antenatal supervision improved obstetric techniques good facilities for blood transfusion and the introduction of antibiotics. But the elimination of deaths and illness resulting from mechanical problems, from toxaemia from placenta praevia and from postpartum haemorrhage now emphasizes other remaining maternal and foetal hazards such as recurrent abortion foetal malformation premature delivery and abruptio placentae the causes of which are in many cases still not evident.

The proper development of the foetus and its secure and efficient implantation in the endometrium are fundamentally dependent on the normal division and metabolism of the cells, those of the actively growing embryo itself those of its exuberant trophoblast and those of the uterine tissues. And the life character and behaviour of these cells like those of all nucleated cells, is in turn dependent on ribonucleic (RNA) and desoxyribonucleic (DNA) acids A deficiency of these results in a disturbance of the normal metabolism of all cells and tissues. Moreover folic acid and its derivatives play a vital part in the synthesis of the essential nucleic acids and are therefore important to cellular

[1] This communication formed the basis of an address given by T. N. A. Jeffcoate at the Annual Meeting of the Finnish Gynaecological Association in Helsinki, May 1965

reproduction This explains why folic acid antagonists such as methotrexate and aminopterin can bring about the destruction of virulent neoplasms.

Pregnancy is characterised by cellular proliferation particularly in the fœtus the trophoblast the uterus the breasts and the hæmopoietic tissues So the pregnant woman has an ever increasing need for nucleic acids and therefore for folates on which nucleic acid synthesis depends. This heightened demand was demonstrated by Chanarin and others (1959) who found a close correlation between folic acid clearance rates and fœtal growth. According to these authors the folic acid clearance rate is already increased by the twelfth week of pregnancy and rises to reach a maximum at the thirty sixth week The highest figures are recorded when the pregnancy is multiple

A deficiency of folic acid always manifests itself first in those tissues growing most rapidly Thus when folic acid antagonists are used in the treatment of chorio-carcinoma their effect shows first in the tumour cells. Thereafter however the tissues affected are the bone marrow (to produce leucopenia and anæmia) the mucosa of the alimentary tract and elsewhere (to produce ulceration of the mouth and anus) and the hair (to cause alopecia) In pregnancy folic acid deficiency is well known to affect hæmopoietic tissues and thus to cause megaloblastic anæmia. But on biological grounds it seems reasonable to postulate that the more actively growing tissues of the fœtus and the trophoblast are more susceptible to such a deficiency than is the bone marrow If this is true then deprivation of folic acid could result in early death of the fœtus (blighted ovum syndrome) abortion fœtal malformation defective placentation and impaired fœtal growth Indeed it is established that experimentally induced folate deficiency in animals has all these effects and moreover it damages the conceptuses before having any obvious ill-effect on the mother (Nelson 1960) Similarly the administration of a folic acid antagonist to pregnant women causes abortion or fœtal malformation (Thiersch 1960)

What follows examines how far these observations and concepts are supported by clinical experience

Material

The enquiry was conducted at Mill Road Maternity Hospital Liverpool during the period August, 1961 to December 1964 This hospital serves a densely populated section of the city one in which housing conditions are poor and the economic, social and educational standards of the community are low The consumption of fresh vegetables by the people of the area is at least 50 per cent below the national average and the protein intake is low Indeed, surveys reported previously (Hibbard B.M., 1964) indicate that 20 per cent[1] of the women delivered in this hospital have a haemoglobin level of less than 70 per cent (Haldane) (10.4 g %) at some stage of pregnancy

During the period of the study 12,070 women were delivered Twenty per cent of these were having their fifth or subsequent baby and 27 per cent of these grande multiparae had husbands out of employment Twenty-one per cent of all patients were either unmarried or had an unemployed husband. An assessment of the folate status was carried out in 3369 cases. Patients specially selected for the investigation included those who had anaemia antepartum haemorrhage multiple pregnancy and an abnormal foetus but, where necessary for statistical evaluations, these were matched with control series of normal women having normal pregnancies and labours.

An additional series of 179 women admitted to the Liverpool Royal Infirmary and Mill Road Maternity Hospital on account of abortion was also investigated and the findings compared with those of a control group of 50 women who were in the early stages of normal pregnancy

THE DIAGNOSIS OF FOLIC ACID DEFICIENCY

Until recently the diagnosis of folic acid deficiency depended on the recognition of the typical morphological changes in the bone marrow or the peripheral blood But these traditional methods have the following disadvantages

BIOCHEMICAL BASIS OF THE FIGLU EXCRETION TEST

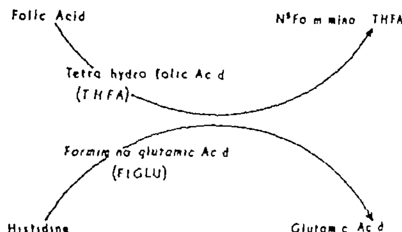


Fig. 1 Biochemical basis of the *FIGLU* excretion test.

(i) *The interpretation of the appearances of blood films* and bone marrow smears varies with individual observers
(ii) Changes in the peripheral blood are not specific and, in any case *only show at a late stage of the disease*
(iii) Megaloblastic erythropoiesis is also a late *manifestation* of *folic acid deficiency*

Other methods of determining the folic acid status *of an indi* vidual include the measurement of serum folate levels and the assessment of folic acid absorption and excretion by chemical, microbiological *and* radio-isotope *techniques However* these techniques are not generally suitable for a large scale investiga tion

The excretion of *formimino-glutamic* acid (*FIGLU*) *in* the urine is now recognised as giving an index of folate metabolism. FIGLU is an intermediate product in the metabolism of the amino-acid *l-histidine*. For the further breakdown of *FIGLU to* glutamic acid, folic acid derivatives are required (Fig 1) In the absence of these folates histidine metabolism is arrested, FIGLU accumu lates and is excreted *in* the urine *in excessive quantities*. FIGLU

may be detected in the urine by a variety of techniques but the majority of these are too complicated laborious or insensitive for the investigation of patients attending antenatal clinics. When using certain techniques many workers have found the FIGLU excretion test to give variable results of questionable significance. However one of us developed a method which depends on high voltage electrophoresis, and which in our hands has been proved both simple and reliable (Hibbard E.D., 1964) The validity of the test as described was established at an early stage of this enquiry and, as the work proceeded there was always good correlation between the amount of FIGLU excreted and the appearances of the bone marrow and the blood. Of 3369 women investigated by the FIGLU excretion test, all had hematological studies performed and 1020 were subjected to marrow biopsy The results of the last in relation to those of the FIGLU excretion test were as follows.

Patients with positive FIGLU tests		–	777
Megaloblastic marrow	310		
Normoblastic marrow	467		
Patients with negative FIGLU tests		–	243
Megaloblastic marrow	0		
Normoblastic marrow	243		

These figures illustrate that the FIGLU excretion test reveals a defect in folate metabolism before this induces obvious changes in the bone marrow That the positive results of the FIGLU tests in women with normoblastic bone marrows were not false was established by the fact that in every such case the test result was reversed by the administration of folic acid supplements.

The FIGLU excretion test gives only an indirect assessment of folic acid status and may be influenced by factors such as intestinal absorption and enzymatic activity in the liver but, because it reflects function, it may in fact be a more reliable index of folate metabolism than tests based on absolute measurements of folate fractions in the serum whole blood or tissues. Thus, in the presence of a metabolic block (such as results from aminopterin therapy) all the clinical signs of folate deficiency including

an excessive excretion of FIGLU are present yet serum folate levels may be high

Results

A FIGLU test is classified as positive when the amount demonstrated in the urine 5 hours after the ingestion of 10 G histidine exceeds 20 μg per ml All tests carried out on normal control female subjects give a figure well below this (Hibbard E.D., 1964)

The incidence of positive tests amongst the series of 3369 pregnant women investigated varied according to the clinical syndromes which they manifested and the details of each group are described later But of all the women in the main series 992 (29.4 per cent) showed a positive FIGLU excretion test at some stage of pregnancy This figure is high since in general, those women investigated were specially selected because of some clinical feature which suggested they were at special risk Nevertheless, if it be assumed that all the women not investigated had negative FIGLU excretion tests the results indicate an incidence of folate deficiency of 8.2 per cent (992 out of the total hospital population of 12,070) Thus must be a minimal figure and a more realistic picture is presented by a special study of a small series of 167 women who had a single pregnancy of 32–36 weeks maturity These were entirely unselected and the group comprised consecutive attenders at the pre-natal clinic Nineteen of these women (11 4 per cent) had positive FIGLU excretion tests and 7 (4.2 per cent) had megaloblastic bone marrows

The conclusion is that not less than 10 per cent of this particular hospital community show evidence of folate deficiency during pregnancy and in nearly half of these the deficiency is so severe and prolonged as to cause megaloblastic erythropoiesis.

CAUSES OF FOLIC ACID DEFICIENCY

A. *Supply and Demand*

Folate deficiency in pregnancy results principally from the increased demand arising as a result of rapid tissue growth The majority of women can meet this demand because their intake

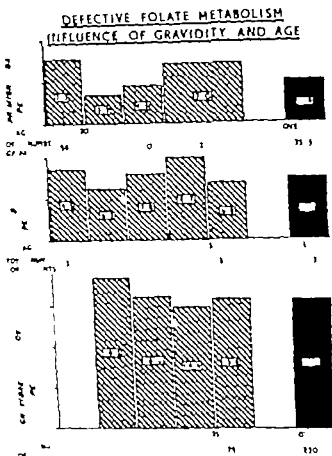


Fig Defective folate metabolism—influence of gravidity and age.

of folic acid in the diet is satisfactory But if the diet is inadequate or if the demand is excessive (as in the case of multiple pregnancy) a deficiency is likely to result.

The body has only limited reserves of folic acid and any stores can soon be exhausted in pregnancy If pregnancies occur in rapid succession time is not available to replace the reserves and, for this and other reasons, folate deficiency is likely to recur in successive pregnancies. The factors governing the supply of and demand for folic acid are illustrated by the following findings.

Table I. *Defective folate metabolism and multiple pregnancy*

	Total	Excreta FIGLU excretion
Singleton pregnancies	167	19 (11.4 %)
Twin pregnancies	113	63 (56 %)
Triplet pregnancies	8	8 (100 %)

1 *Age and Gravidity*

The incidence of defective folate metabolism is closely related to the number of previous pregnancies (Fig 2) In our series it ranged from 4 9 per cent in primigravidæ through 7.4 per cent in gravidæ 2–4 to 15.7 per cent in gravidæ 5 and over The grande multipara is 3 times as likely as the primigravida to suffer from folate deficiency

There were no major variations with age although the younger mothers in each parity group appear to be at a slightly greater risk This is especially true of young primigravidæ of which in our series a high proportion were unmarried, had a poor diet and attended late for antenatal care The somewhat higher incidence of folic acid deficiency in the elderly primigravidæ is not so easy to explain Seventeen out of 241 aged 30 years and over and 8 out of 64 aged 35 years and over had positive FIGLU excretion tests. The numbers are small but if they are significant it may be that in this particular community elderly primigravidity is so unusual as to suggest that the women involved had an abnormal socio-economic background resulting in poor nutritional standards In certain cases they were known social misfits. The effect of youth on the occurrence of folate deficiency amongst multigravidæ can be accounted for by the fact that the younger women had pregnancies more closely spaced with insufficient intervals to build up folic acid reserves

2 *Multiple Pregnancy*

The influence of an exceptional demand for folic acid is illustrated by the findings in cases of multiple pregnancy (Table I)

Table II. *Defective folate metabolism—time of diagnosis*

Gestation (Weeks)	Patients investigated	Excessive FIGLU excretion — first positive test
≤ 12	107	29 (27 %)
13–20	339	70 (20.5 %)
21–28	702	177 (25.2 %)
29–36	2162	451 (20.9 %)
> 36	1025	267 (26 %)

Totals exclude subsequent tests on any patient found to have a positive test at an earlier period, but include negative tests in patients who subsequently had positive tests. The figures are biased by selection: for example, most tests before 13 weeks gestation were carried out because of abortion or a history of defective folate metabolism in a previous pregnancy. On the other hand many patients with positive tests after 36 weeks gestation were admitted as emergency cases and had had little or no prior antenatal care.

Out of 113 women with twin pregnancies who were studied 63 had positive FIGLU excretion tests. This incidence of 56 per cent is 5 times greater than that expected. Eight women carrying triplets all had evidence of defective folate metabolism.

B. *Errors in Absorption and Metabolism*

Because overt megaloblastic anaemia is a disease of late pregnancy and the puerperium, it is often assumed that folic acid deficiency only appears as a result of the ever increasing demands of the enlarging foetus. In fact, many women show positive FIGLU tests in early pregnancy long before the foetus and the trophoblast are sufficiently developed to utilize large amounts of folic acid. As this became recognised all patients with a haemoglobin level below 75 per cent (Haldane) (11.1 g %) were investigated irrespective of the period of gestation. Table II shows the distribution of all positive reactions according to the duration of pregnancy. In the interpretation of these findings it has to be recognised that many women did not attend until their pregnancy was far advanced. Despite this 16 per cent of all the FIGLU positive patients were less than 24 weeks pregnant, and more than 50 per cent less than 32 weeks pregnant at the time of the test.

The detection of defective folate metabolism early in pregnancy before the fœtal demands are great, suggests that in some cases the woman is unable to absorb or utilize folic acid. It is well established that folic acid deficiency can be a feature of malabsorption syndromes in the non-pregnant individual It can also result from certain types of bacterial and helminthic infestations of the gut in which circumstances the parasites take up folic acid at the expense of the host.

Even if folates are available in adequate amounts and are absorbed they still may not be utilized Folic acid itself is biologically inactive and can be effective to the tissues only after conversion into folinic acid by enzymatic action This type of defect is seen when folic acid antagonists are used in the treatment of malignant disease A partial inhibition of folic acid metabolism can also attend the administration of anti-convulsant drugs (as for epilepsy) barbiturates and possibly certain long acting sulphonamides all of which are sometimes given to pregnant women

This raises the question as to whether the state of pregnancy itself can modify folate metabolism Other investigators have demonstrated that it can Thus Hansen and Klewesahl Palm (1963) noted major alterations in the folic acid clearance rate as early as the seventh and eighth weeks of pregnancy Moreover when investigating serum folate levels in women with megaloblastic anæmia of pregnancy Ball and Giles (1964) found that the labile folate fraction which may correspond to the metabolically active folinic acid is consistently low although the total serum folate level is frequently within normal limits. These observations support the concept of an intrinsic metabolic defect in certain patients. In this the hepatic enzyme system is probably involved and the responsible factor may be the high level of steroids in circulation during pregnancy (Girdwood, 1953) Œstrogens in particular modify liver function (Tindall and Beazley 1965) and they also stimulate purine metabolism for which a supply of folate is necessary It is therefore necessary to recognise that a folate deficiency can occur in early as

Seven pregnant women receiving phenytoin were investigated by us and 6 had abnormal FIGLU excretion tests.

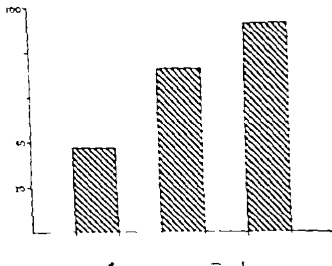


Fig. 3 Defective folate metabolism. Reversion to normal FIGLU excretion following oral therapy (Folic Acid 5 mgm. t.d.)

well as in late pregnancy and that when it does it probably reflects an error in absorption or utilization rather than an intake inadequate to meet bodily requirements.

In certain cases more than one mechanism operates to cause the disorder. Thus one of the effects of simple dietetic deficiency is atrophy of the mucosa of the gastro-intestinal tract. This mucosa is an actively reproducing tissue its replacement taking place at the rate of approximately 50 g. per 24 hours (Witts, 1957). Even a minor shortage of folates may interfere with this process with the result that the alimentary tract's capacity to absorb folic acid becomes impaired. So it is not surprising that the oral administration of folic acid does not always remedy an established folate deficiency. The response of most affected pregnant women to large doses of folic acid is rapid, as judged by

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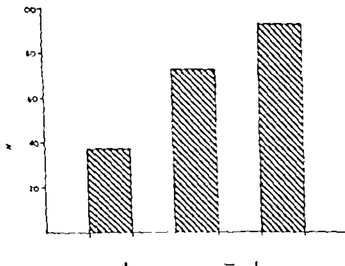


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FIGLU excretion tests and hæmatological criteria (Fig 3) But amongst 444 women in this series with a proved deficiency there were 34 (7 7 per cent) who failed to respond to oral medication (15 mgm daily) within 3 weeks but who were dramatically cured by subsequent parenteral therapy

So it would appear that in the majority of cases the altered metabolism of folates in pregnancy can be corrected or surmounted providing folic acid is administered in adequate quantity and by an effective route

Recurrence of Defective Folate Metabolism

Irrespective of the causal mechanism operating during pregnancy all evidence of folate deficiency usually clears soon after delivery As many as possible of our patients who showed positive FIGLU excretion tests in late pregnancy and the puerperium were re-tested in the subsequent weeks All these were treated and in most cases negative tests were obtained after very short intervals but there were some who required treatment for 6 weeks and a few for 12 weeks, before showing normal FIGLU excretion

In 122 cases the woman was investigated in a subsequent pregnancy and in 82 of these (67 per cent) the FIGLU excretion test again became positive Twenty-six of the 122 women, because of the findings in the previous pregnancy had had folic acid prescribed prophylactically in the succeeding pregnancy Despite this 13 (50 per cent) had abnormal FIGLU excretion tests

It would seem that in many woman the available reserves of folates in the body are at the borderline of normality and safety A deficiency cannot usually be demonstrated between pregnancies but it shows up almost immediately conception occurs.

Clinical Manifestations

1 *Pregnancy anæmia*

The classical effect of a folic acid deficiency in pregnancy is megaloblastic anæmia Among our hospital population there

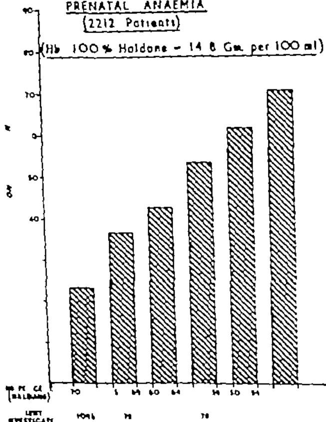


Fig 4 Excessive FIGLU excretion and pre-natal anaemia

were 2212 women whose haemoglobin level was less than 75 per cent (Haldane) or 11 1 G per cent at some stage of pregnancy and 713 (32·3 per cent) of these had a positive FIGLU excretion test. More than 500 of the anaemic patients also had marrow studies. But the findings agreed with the FIGLU test so consistently that marrow biopsy was omitted in the later stages of the enquiry unless specifically indicated. In not one case was evidence of megaloblastic erythropoiesis found without the

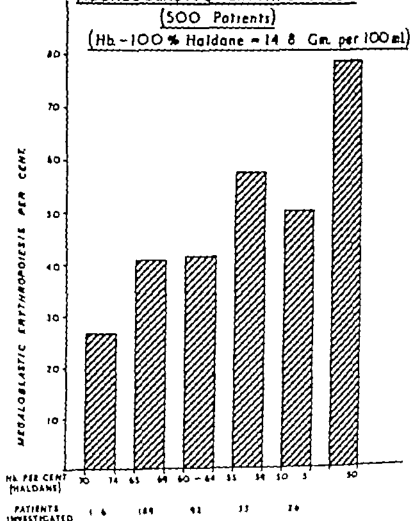


Fig 5. Megaloblastic erythropoiesis in women with prenatal anaemia associated with excessive FIGLU excretion

FIGLU excretion test being positive In certain cases serum iron estimations were also made and the results indicated that many women suffered from iron as well as folic acid deficiency In this respect it has long been recognised that cases of what was formerly regarded as iron resistant hypochromic anaemia of pregnancy frequently respond to treatment with folic acid There is also evidence that a chronic iron deficiency can ultimately lead to folic acid deficiency (Chanarin and others, 1965) So in

practice the picture can be one which is confused and which reflects more than one deficiency

The more severe the anaemia the more likely is the FIGLU test to be positive and the more likely is the bone marrow to show megaloblastic characteristics Thus as is indicated in Figs. 4 and 5, a positive FIGLU test was found in 23 1 per cent cases when the haemoglobin level was between 70 and 74 per cent, and in 70.8 per cent cases when the haemoglobin level was less than 50 per cent. Amongst women with a haemoglobin level of 70 to 74 per cent and showing a positive FIGLU test, megaloblastic erythropoiesis was discovered in 26 7 per cent. When the haemoglobin level was below 50 per cent and the FIGLU test was positive megaloblastic erythropoiesis was diagnosed in 78.6 per cent cases. From our observations it can also be calculated that if all anaemic women had been subjected to bone marrow biopsy and not merely those with an abnormal FIGLU excretion evidence of megaloblastic erythropoiesis would have been found in 56 per cent cases when their haemoglobin level was below 50 per cent, and in 6 2 per cent cases when the level was between 70 and 74 per cent.

These findings all go to emphasize a point made previously namely that folic acid deficiency is revealed at an earlier stage by the FIGLU excretion test and that histological changes in the bone marrow appear later clinical anaemia is often the last of the signs. So the more severe the anaemia the more likely are the other tests to be positive

2. *General ill-health*

Following the last observation it needs to be recognised that women with defective folate metabolism often have signs or symptoms of it before they become anaemic. These are of a general character and can be missed unless careful watch is kept. They include tiredness and listlessness anorexia, and changes in the scalp hair such as loss of lustre and a tendency to break. The exhaustion and lack of energy manifest themselves in a characteristic facies. Not all such minor and vague complaints can be attributed to folate deficiency but in many cases they disappear following folic acid medication.

3. *Abruptio Placentæ*

The evidence showing an important relationship between abruptio placentæ and defective folate metabolism was reviewed previously (Hibbard and Hibbard, 1963; Hibbard, B. M., *1964)*. *Coyle and Geoghegan (1962) reported that 35 out* of 77 (45 per cent) patients with "accidental antepartum hæmorrhage" showed megaloblastic erythropoiesis. In a similar investigation, but adopting more rigid criteria for the diagnosis of abruptio placentæ we found megaloblastic changes in the bone marrow in 64 per cent of patients suffering from this disease (Hibbard and Hibbard, 1963). Since bone marrow changes take some time to develop it can confidently be assumed that they were present before placental detachment occurred. Indeed in our experience, women known to have megaloblastic changes in the bone marrow have a 5 times greater than expected chance of developing abruptio placentæ.

In the period under review, FIGLU excretion tests were carried out on 161 patients suffering from proved abruptio placentæ and abnormal results were obtained in 157 cases (97.5 per cent). Moreover, at least 2 of the 4 patients showing a normal excretion of FIGLU had been taking folic acid prior to the investigation. In 42 (26 per cent) cases the test was known to be abnormal prior to the initial hæmorrhage, the patients having been investigated for some other reason; in the remainder the test was carried out a short time after the occurrence of the hæmorrhage.

Patients with antepartum hæmorrhage from other causes were also investigated. A positive FIGLU excretion test was found in 16 out of 68 cases of placenta prævia (24 per cent). This rate is at least twice as great as that of overall hospital population, but many women with placenta prævia are of high parity and therefore are more likely to show evidence of folate deficiency.

Three hundred and forty patients suffering from antepartum hæmorrhage of uncertain origin were also investigated and excessive FIGLU excretion was detected in 148 (43.5 per cent). This high incidence is almost certainly explained by the inclusion in this group of a number of cases of abruptio placentæ in which

the strict criteria adopted for the diagnosis of abruption were not satisfied.

It may be added that women who suffer severe post-partum haemorrhage do not show a positive FIGLU excretion test (Hibbard B. M., 1964) thus proving that such a finding is not the result of haemorrhage.

The close association between folic acid deficiency and abruptio placentae is emphasized by other well established observations. For example abruptio placentae like folate deficiency is a disease of the grande multipara tends to recur in successive pregnancies, and is more likely in women of poor social and economic status. The probable explanation is that a defect in folate metabolism hinders the growth of trophoblast in the early days of pregnancy and makes for faulty chorio-decidual relations. For the same reason the babies in these cases are unusually small and weakly The insecurity of the attachment of the placenta in association with folate deficiency does not mean that abruption in late pregnancy is inevitable It is a strong predisposing factor but the actual occurrence probably requires a precipitating factor This may be hypertension in a small proportion of cases but pre-eclampsia and chronic hypertension are not important causes of abruptio placentae Hibbard B M. 1962 Hibbard, and Hibbard, 1963) It is tempting to postulate that uterine muscle activity sometimes determines the placental detachment, the evidence being that not less than 40 per cent women admitted to hospital suffering from abruptio placentae are already in labour

This concept means that the chorio-decidual defect which leads ultimately to abruptio placentae occurs very early in pregnancy Moreover once the damage has been done it probably cannot be reversed by administering folic acid Thus the majority of the 42 women in whom abnormal FIGLU excretion was demonstrated before the occurrence of abruptio placentae had been given folic acid supplements as soon as the result of the test was known Moreover it is known that, in 1 cases this had the effect of reversing the test findings before haemorrhage occurred. But the correction of the folate metabolism in these women did not prevent subsequent detachment of the placenta

It may also be noted that multiple pregnancy is not significantly complicated by abruptio placentæ even though it commonly causes depletion of the folates in the body This is presumably because the deficiency in these cases is entirely the result of fœtal demands and occurs late in pregnancy long after placentation is well established

4 *Previous obstetric history*

Multigravidæ who suffer from abruptio placentæ often have "a bad obstetric history So at an early stage in this enquiry a consecutive series of 469 pregnant women whose FIGLU excretion test was positive were compared with a control series of 359 women unselected except for matching for age and parity The findings reported previously (Hibbard B M. 1964) showed that women with evidence of folate deficiency in pregnancy had had more abortions more premature labours, more fœtal malformations as well as more pregnancies complicated by antepartum hæmorrhage than was statistically expected. Indeed the number of previous unsuccessful pregnancies—those failing to result in a normal living child—was 29 per cent higher in the folate deficient group than in the control group of patients.

As a result of these findings special studies of women who aborted or produced a malformed child were instituted and are still in progress Already however the findings are such as to be of interest.

5. *Abortion*

All women admitted to the Liverpool Royal Infirmary and Mill Road Maternity Hospital suffering from threatened or inevitable abortion were studied. Those cases in which there was a known probable cause for the abortion bicornute uterus and incompetent cervix for example were excluded. There remained 179 patients who aborted for no apparent reason and these were subjected to a FIGLU excretion test before or immediately after the termination of the pregnancy In Table III the findings are compared with those obtained from tests carried out on 50 control subjects matched for the period of gestation From this Table

Table III. *Defective folate metabolism in patients suffering from abortion*

	Total	Excessive FIGLU excretion
Control cases	50	3 (6 %)
Isolated abortion	3	42 (32 %)
2 or more consecutiv abortions	48	29 (60.5 %)

It will be seen that the FIGLU excretion test was positive in 32 per cent of women having an isolated abortion and in only 3 per cent of the control pregnant patients. In the cases of women having a second or subsequent consecutive abortion the percentage of abnormal tests rose to 60.5. Abortion can have many causes but the findings suggest that one of these is defective folate metabolism in the mother In women having a succession of abortions the chance of this aetiological factor being present is high. The patients investigated were a selected group in that they were drawn mainly from those who had previously attended an infertility clinic and were therefore of inferior reproductive capacity Moreover the mere demonstration of the presence of a defect in folate metabolism does not exclude other aetiological factors. Such a defect may be merely an index of a more general metabolic or dietary inadequacy and may be only one of several adverse factors all of which combine to kill the foetus or cause abortion

6 *Malformation of the Foetus*

In order to be reasonably certain that defective folate metabolism is a significant cause of foetal malformation it is desirable to demonstrate that the defect is present at the time of relevant organogenesis. This has been done experimentally in animals and also in women who have been given folic acid antagonists during the early stages of pregnancy (Thiersch, 1960 Goetsch, 1962 de Alvarez, 1962) From such observations it appears that a deficiency in folic acid is especially liable to result in gross foetal deformity and especially a malformation attributable to a fusion defect.

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showed an excessive excretion of FIGLU as compared with 8 (15 per cent) in the control group The nature of the foetal mal formations was diverse but anencephaly or iniencephaly were present in 22 cases and it is notable that this is a type of defect commonly produced when pregnant animals are deprived of folic acid. Thirteen of the mothers (59 per cent) delivering anencepha lic or iniencephalic monsters showed positive FIGLU excretion tests.

As in the case of abortion it is unjustifiable to conclude that there is a direct cause and effect relationship between disturbed folate metabolism and foetal malformation. Again there may be multiple aetiological factors with a possible interplay between genetic and environmental influences. But the evidence so far available suggests that women who deliver abnormal babies often offer the foetus an unsatisfactory environment during late preg nancy and may therefore do so in the weeks immediately follow ing conception

Prevention and Treatment

When a folate deficiency is demonstrated in any patient there is a clear indication for administering folic acid. The dose ordi narily employed, 5 mgm. t. d. s. by mouth, greatly exceeds the calculated requirements of the normal pregnant women but, bear ing in mind the possible need to over-ride impaired absorption by the alimentary tract, is conducive to obtaining a rapid and satis factory response. Moreover the frequent coincident occurrence of an iron deficiency makes it prudent to give iron supplements to all women receiving folic acid therapy Even if an iron defici ency is not demonstrated initially it is likely that a sudden increase in haemopoietic activity following folic acid administration will deplete the iron reserves. Vitamin C plays an important part in folate metabolism and it is notable that many of the foods rich in folic acid also contribute a significant amount of ascorbic acid to the diet. It is therefore wise to include ascorbic acid in the therapy of folate deficient patients. All affected patients in our hospital are treated with a compound tablet containing these three factors. Even this does not supply all the metabolic needs for cell

Table IV *Defective folate metabolism in mothers of malformed infants*

	Total	Excessive FIGLU excretion
Mothers of malformed infants	54	35 (65 %)
Mothers of normal infants	54	8 (15 %)

Clinical studies are handicapped by the fact that it is impracticable to estimate the folic acid status of large numbers of women while they are in the early stages of pregnancy Any evidence about the part played by abnormal folate metabolism in the production of foetal malformations has therefore to be indirect and to involve the assumption that conditions obtaining in late pregnancy reflect those present at or soon after the time of conception

Amongst the women who delivered in Mill Road Maternity Hospital and who were investigated 992 showed excessive FIGLU excretion at some time during pregnancy Of these 36 (3 6 per cent) produced a baby which had major congenital malformations Amongst the -377 patients with a normal FIGLU excretion test there were 1 5 per cent who delivered malformed infants The difference in incidence is significant but does not permit a firm conclusion that defective folate metabolism was in itself responsible for the increased number of malformations Other selective factors may be involved. For example a positive FIGLU test is more likely in women who are relatively old highly parous or anaemic and any of these factors could also be operating in causing congenital defects in the foetus.

Because of the above considerations a special study of the problem was commenced in 1964 This involved the deliberate search for women delivering a grossly malformed baby and to control the findings by matching each case with a woman of similar age parity social status and period of gestation carrying or delivering a normal child The results in the first 54 cases of this new series are described elsewhere (Hibbard and Smithells 1965) but can be summarised as follows

Thirty-five (65 per cent) of the women with a malformed baby

before she seeks medical advice. In most cases this damage probably can not be corrected, even though the causal folate deficiency is made good later in pregnancy. In those cases in which defective folate metabolism appears likely to affect the foetus and its trophoblast, that is in those women with a previous history of unexplained recurrent abortion and of abruptio placentae treatment must needs commence before conception occurs. That this argument is valid is supported by the experience of Martin and others (1965) who administered folic acid before conception in cases of recurrent abortion with some degree of success.

Although the emphasis here and elsewhere is placed on folic acid because it is fundamental to the synthesis of nucleic acids, it has to be recognised that the woman deficient in folates is probably deficient in other factors which are important to the health of cells and tissues. The best means of raising reproductive efficiency and of preventing the pregnancy complications discussed in this communication is to ensure that women are in good general nutritional state before as well as during pregnancy. Good nutrition is in large measure dependent on factors of housing, education and economic status and so it is that all these factors combine to determine foetal and maternal mortality and morbidity.

The perinatal mortality rate and the incidence of abortion and premature labour are in most Western communities directly related to social grading, being lowest in those women who live in the most favourable circumstances. Moreover as the general well being of a community improves serious obstetrical complications such as abruptio placentae tend to disappear or to become less frequent. But even when all women have the best dietary habits and living standards it seems likely that there will remain some who will continue to abort, to have malformed children and to suffer abruptio placentae because of a folate deficiency. In such cases the error will be an inherent metabolic one, possibly genetically determined, for which a satisfactory treatment has still to be discovered.

and tissue growth. Proteins, for example, are also essential and are not adequately represented in the diet of a large fraction of our hospital population.

Bearing in mind the frequency of pregnancy anæmia and of folate deficiency, it is often recommended that folic acid should be administered routinely to all pregnant women as a prophylactic measure. The risk of inadvertently treating a case of true Addisonian anæmia which this practice involves is extremely small providing reasonable clinical precautions are taken. Routine and blind prophylactic therapy may be justified when facilities for antenatal supervision and investigation of patients are limited and when the population served is known to have poor dietary habits. It is certainly in such circumstances desirable for all patients at known increased risk, namely those of high parity, those with multiple pregnancy and those with a history of severe anæmia or of defective folate metabolism in a previous pregnancy. But in general it is preferable not to administer folic acid except when a need for it is clearly demonstrated by full investigation of the case. Apart from any other consideration it is our experience that the routine prescribing of treatment as a prophylactic measure fails to achieve its object because many pregnant women do not obey instructions and omit to take the tablets. Unless the patients knows that treatment is based on the result of hæmatological and other tests she remains unconvinced of the need for it.

When folic acid is administered orally to remedy a proven deficiency state it proves ineffective in approximately 8 per cent cases. In these malabsorption (sometimes consequent upon the deficiency state) makes it necessary to give the preparation by intramuscular injection at least until the damage to the alimentary mucosa is repaired.

The administration of folic acid together with iron and ascorbic acid prevents or corrects pregnancy anæmia in the great majority of women. But this treatment, when applied to the woman already pregnant, does not necessarily prevent abortion, fœtal malformation and antepartum hæmorrhage. This is because, as suggested earlier, these happenings, if related to defective folate metabolism, reflect damage to embryonic tissues soon after conception, before the patient is confident that she is pregnant and

has defective folate metabolism has a 5 times increased chance of abruptio placentae in later pregnancy

Amongst 179 women who suffered abortion for which there was not a clear cause 32 per cent had an abnormal FIGLU excretion test. The comparable figure for a control group of women with normal pregnancies of similar duration is 3 per cent. In women having a second or subsequent consecutive abortion the chances of finding evidence of defective folate metabolism is 60 per cent.

Of 54 women delivering a malformed baby 65 per cent had an abnormal FIGLU excretion test as compared with 15 per cent in the control group

The principal cause of defective folate metabolism is an intake inadequate to satisfy the needs of the mother and child. But in some cases the intake is satisfactory but absorption is poor or the subsequent metabolism of folic acid to folinic acid is disturbed. Faulty absorption can itself result from folic acid deficiency

The administration of folic acid (with iron and vitamin C) to the pregnant woman corrects or prevents megaloblastic anaemia but it does not protect her from abortion from producing a malformed foetus or from abruptio placentae. Such happenings may be related to damage which occurs in the early days of placentation and this damage cannot be reversed To avoid these accidents of pregnancy it appears to be necessary to provide the conceptus with an adequate nutritional environment in which other factors as well as folic acid are concerned, before and at the time of conception

Acknowledgement

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SUMMARY

All cellular life and function depends on R N A and D N A, and folic acid is essential to the synthesis of these nucleic acids. The more active the cellular proliferation and tissue growth the greater the need for folic acid

When in the case of a pregnant woman the intake of folic acid is inadequate or when folate metabolism is faulty the tissues which suffer most are those of the trophoblast the fœtus, the maternal bone marrow and the mucosa of the maternal alimentary tract

Defective folate metabolism therefore not only results in megaloblastic anæmia of pregnancy but is concerned in the ætiology of abruptio placentæ and of certain cases of abortion and fœtal malformation

Statistical evidence in support of these concepts is presented and is based on the survey of 17 050 women delivered in a maternity hospital which serves a poor class community in Liverpool.

Of these women 3369 were investigated from the standpoint of their folic acid status. All had *FIGLU* excretion tests and peripheral blood examinations carried out and 1020 were subjected to marrow biopsy

The FIGLU excretion test, providing a satisfactory technique is used is not only a reliable guide to folate metabolism but is a more sensitive index than are cytological changes in the marrow and blood

Not less than 10 per cent of the pregnant women attending the hospital had evidence of defective folate metabolism and it showed a strong tendency to recur in successive pregnancies. The finding of a deficiency is most likely in women of high parity and in those with multiple pregnancy A grande multipara is 3 times as likely to suffer from this condition as is a primigravida Fifty per cent of women carrying twins have evidence of folate deficiency

Among 161 women suffering from proved abruptio placentæ 157 showed a positive *FIGLU* excretion test and 64 per cent had megaloblastic changes in the bone marrow A woman who

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MYOMETRIAL BLOOD FLOW IN PREGNANCY MEASURED WITH XENON^{133}

BY

H. LYSGAARD AND H. LEFÈVRE

Foetal wellbeing depends on a satisfactory environment. This is influenced by among other factors, the maternal blood supply to the placenta With the development of new methods for investigating physiological functions interest in measurement of uterine blood flow has increased.

In 1953 Browne and Veall demonstrated that it was possible to obtain a measure of the placental blood flow by using Na^{24} These authors found a considerable decrease of the NA^{24} clearance in patients with hypertension and preeclampsia. Subsequent authors, including Dixon *et al.* 1963 Johnson and Clayton 1957 Moore and Myerscough, 1957 Morris *et al.* 1955 Weis *et al* 1958 and Wright *et al* 1958 have also found a reduced Na^{24} clearance in the myometrium in certain abnormal pregnancies. Dixon *et al* found that the clinical findings were better correlated with Na^{24} clearance from the placenta than with the clearance from the myometrium. They pointed out that the range of values of Na clearance in the myometrium was very wide Unfortunately a direct determination of placental clearance is possible only when the placenta is situated anteriorly in the uterus. As Na is not freely diffusible in the tissues the values found by a Na^{24} clearance test are inapplicable as a measure of the absolute blood flow in the placenta or in the myometrium.

Lassen, Lindbjerg and Munck (1964) demonstrated that

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an abdominal area having a diameter of about 20 cm. The rate at which Xenon disappeared from the tissues was measured and these observations were recorded on the ordinate in a semilog arithmic system. The abscissa showed the time in minutes (cf Fig. 1)

On the basis of this curve the biological half-life (T½) is found. As the disappearance rate depends exclusively upon the blood flow in the tissues, the following formula (Munck et al 1964) may be used for the calculation $\text{MyBF} = \frac{0.693}{T\frac{1}{2}} \times \lambda \times 100$ ml/100 g tissue/minute, where λ is the partition coefficient between tissue and blood for Xenon λ is taken to be 0.7 which Conn (1961) found to apply to striated muscles in dogs.

Owing to the great solubility of Xenon in air no aspiration was done to make sure that the needle had not entered the amniotic sac a blood vessel or the placenta as the slightest ad mixture of air would have given erroneous curves.

A total of 113 measurements were performed on 85 patients.

Results

The results are shown in Table I From 46 tests on 34 normal pregnant women the mean MyBF was found to be 12.7 ml/100 g/min. with a range from 2 to 32 ml The birth weight averaged 3690 g for 27 cases in which it was known

Eight patients with pre-eclampsia had a total of 12 measurements. The mean MyBF was found to be 12.7 ml/100 g/min with a range from 3 to 26 ml. The birth weight averaged 3200 g for 5 cases in which it was known Most of these patients had very mild pre-eclampsia In two patients with severe pre-eclampsia the mean MyBF was only 7 ml.

In 8 patients with clinical signs of placental insufficiency 10 measurements were done The average MyBF was 10 ml/100 g min with a range from 2 to 26 ml The birth weight averaged 2793 g for 7 cases in which it was known

Seven measurements were performed on 3 patients with hyper tension without proteinuria. The mean MyBF was 7.4 ml/100 g/min. with a range from 3 to 10 ml The birth weights were known in 2 of these cases and were 3000 and 4000 g respectively

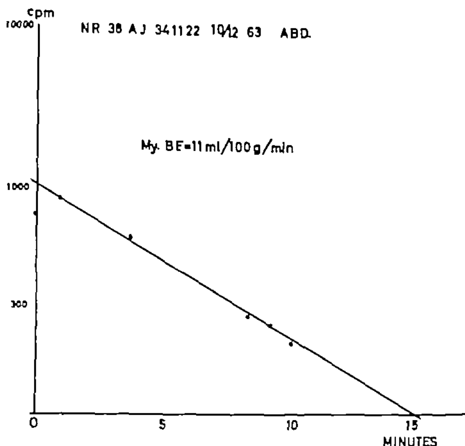


Fig. 1

the inert radioactive gas Xenon is well-suited for measuring the blood flow in the skeletal muscles. It was reasonable therefore to use this substance for determining myometrial blood flow (MyBF). As with Na the preliminary results (Munck *et al.* 1964) showed a marked scatter but a considerably reduced MyBF was evident in abnormal pregnancies.

Method and Material

With a No. 25 needle 0.1 ml isotonic NaCl containing about 50 μC Xenon[133] was injected through the abdominal wall into the myometrium of pregnant women in the last trimester. A scintillation detector coupled to an automatic scaler and printer was placed about 10 cm above the site of injection thus covering

an abdominal area having a diameter of about 20 cm. The rate at which Xenon disappeared from the tissues was measured and these observations were recorded on the ordinate in a semilog arithmic system. The abscissa showed the time in minutes (*cf* Fig 1)

On the basis of this curve the biological half-life (T½) is found. As the disappearance rate depends exclusively upon the blood flow in the tissues, the following formula (Munck *et al.* 1964) may be used for the calculation $MyBF = \frac{0.693}{T\frac{1}{2}} \times \lambda \times$ 100 ml/100 g tissue/minute where λ is the partition coefficient between tissue and blood for Xenon. λ is taken to be 0.7 which Conn (1961) found to apply to striated muscles in dogs.

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In 8 patients with clinical signs of placental insufficiency 10 measurements were done The average MyBF was 10 ml/100 g/min. with a range from 2 to 28 ml The birth weight averaged 2793 g for 7 cases in which it was known.

Seven measurements were performed on 3 patients with hyper tension without proteinuria The mean MyBF was 7.4 ml/100 g/min. with a range from 3 to 10 ml. The birth weights were known in 2 of these cases and were 3000 and 4000 g respectively

Table I.

Type of Pregnancy	No of Measurements	No of Patients	MyBF Mean	MyBF Range	No of Infants	Average Birth Weight
Normal	46	34	12.7	2–32	27	3690
Pre-eclampsia	12	8	12.7	3–26	5	3200
Placental insufficiency	10	8	10.0	2–26	7	2793
Hypertension without proteinuria	7	3	7.4	3–10	2	3600
Premature rupture of membranes	4	4	9.2	5–16	5	2975
Chronic renal disease	3	3	14.3	12–17	2	3225
Twins	3	3	7 3	5–10	6	2700
Prolonged pregnancy	2	2	9.0	(8–10)	2	3875
Patients with foetal death	2		7.0	(7–7)		
Patients with foetal death during labour	2	2	7 5	(5 10)		
Measured during labour	3	3	10.7	(8–14)		
Measured after delivery	2	2	13 0	([illegible]–24)		
Measurement failed	17	15				

Isolated measurements were done on 4 patients with premature rupture of the membranes. The mean MyBF was 9.2 ml/100 g/min with a range from 5 to 16 ml. One of the patients was carrying twins The average birth weight of these infants was 2975 g

Three patients with chronic renal disorders each had a single measurement. The mean MyBF was 14 3 ml/100 g/min with a range from 12 to 17 ml

Three patients carrying twins each had a single measurement. The mean MyBF was 7 3 ml/100 g/min with a range from 5 to 10 ml The average birth weight of the 6 infants was 2700 g

Two patients with prolonged pregnancies (more than 42 weeks) had single measurements The MyBF was 8 and 10 ml/100 g/min respectively

Two patients with intrauterine foetal death had single measurements. The MyBF was 7 and 7 ml./100 g/min respectively

In 2 cases the foetuses died during labour The MyBF had previously been measured as 5 and 10 ml/100 g/min

The average of the 45 measurements in abnormal pregnancies is 10 ml/100 g/min

In addition 3 measurements were carried out during labour The mean MyBF was found to be 10 7 ml/100 g/min. with a range from 8 to 14 ml.

Two patients were investigated shortly after delivery The MyBF was 2 and 26 ml/100 g/min respectively

Seventeen investigations on 15 patients failed partly for technical reasons and partly because the patients moved too much during the test.

Discussion

The object of this study was to determine the absolute myometrial blood flow in pregnant women at or close to term. The mean MyBF was approximately 12–13 ml/100 g/min. with a range from 2–32 ml Using the N_2O method during operation, Assali et al (1960) found the uterine blood flow in pregnant women to be between 6 6 and 12 ml/100 g/min By the same method also employed during operation, Metcalfe et al. (1955) found the uterine blood flow to be 12.4 ml/100 g/min with a range from 6.4–22 ml. By means of an electro-magnetic flow meter Assali et al (1960) during operation found the uterine blood flow to be around 9 ml/100 g/min The present measurements with Xenon were performed under physiological conditions and with very slight disturbance to the patient who was not in any case premedicated or otherwise drugged.

Furthermore, it was the intention to ascertain whether the determination of the MyBF could give an index of the foetal prognosis in abnormal pregnancies. Of course the numbers are so far too small to permit any decisive conclusions and the great scatter of the results makes it difficult to evaluate each individual measurement There are a number of possible causes for this scatter

It may be due to an erroneous injection (for example as no aspiration was done) the depot of Xenon may be deposited into the subcutaneous tissue amniotic sac or a vessel. However measurements of Na clearance in which aspiration has been done have shown an equally great scatter It may be imagined that

the distance from the site of injection to the placenta may be a factor Varying activity in the individual muscular segments is also a possible explanation Lastly the scatter may perhaps be due the Xenon depots being injected at a different depth in the myometrium the flow in the vicinity of the serosa perhaps being different from that in the vicinity of the endometrium For these reasons it would be important to be able to make sure that the distance from the amniotic sac was the same each time as this would give more comparable values

The radiation dose to the fœtus and the gonads was calculated as approx 0.2 millirad which is about 0 2 per cent of the dosage received in a general survey of the abdomen and approx 0 5 per cent of the activity to which the fœtus and gonads are exposed when the investigation is performed with Na

CONCLUSION and SUMMARY

1 The mean myometrial blood flow was found to be between 12 and 13 ml/100 g/min with a range from 2–32 ml in normal pregnant women in the last trimester

2 The findings indicate a reduction of MyBF in certain abnormal pregnancies

3 The radiation dose is very low

The authors submit the results of 113 measurements of the myometrial blood flow in pregnant women in the last trimester The investigations were performed with Xenon ^{133} deposited directly into the myometrium by the transabdominal route

In 46 measurements on women with normal pregnancies the mean myometrial blood flow was found to be 12 7 ml/100 g/min with a range from 2–32 ml

In 45 measurements on women with abnormal pregnancies the mean blood flow was found to be 10 ml/100 g/min with a range from 2 to 26 ml

Probably this marked scatter is due to the injected material being deposited at different depths in the myometrium and at varying distances from the placenta

The radiation dose to the fœtus and gonads is only about 0 5 per cent of that received when the measurement is done with Na

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nant women with varying degrees of toxemia and also compared them with normal pregnant women. We also compared our results with other published series describing the diurnal variations in healthy subjects. A possible similarity between the cycles of the pregnant groups and of patients with congestive heart failure or hepatic cirrhosis was postulated.

Material

Fifty-five pregnant women were investigated during the tenth lunar month of pregnancy. In twenty-nine cases the pregnancy was normal and in six cases there was oedema present without albuminuria or hypertension. The remaining twenty cases were all toxemic. Five of these twenty had hypertension of pregnancy the blood pressure being greater than 160/110 and they also had oedema. Eleven had mild pre-eclampsia with albuminuria. Four patients had severe pre-eclampsia with subjective symptoms (based on the classification of the toxemias of pregnancy by the American Committee on Maternal Welfare).

Method

The investigation was carried out as soon as possible after admission, usually the following day. No diuretic or hypotensive drugs had been administered prior to admission and none were given during the investigation. Diet consisted of ordinary hospital food and the toxemic patients were not given a low salt diet until after the investigation was completed. Meals were served at 7, 9, 16 and 19 o'clock. The first and last meals consisted of light food and the others of more substantial food. During the investigation the patients were confined to bed. Each patient had an in-dwelling catheter in the bladder which was drained every two hours and the volume of urine was measured. The urinary potassium and sodium contents were estimated using the flame photometer and the excretion of these electrolytes was determined. Twelve specimens of urine were thus collected from every patient and the sodium and potassium contents determined every two hours. The patients were divided into five groups to simplify the analysis of the results.

Pystynen Paavo and Pankamaa Paavo Acta obst. et gynec. scandinav 44 458 1965
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DIURNAL VARIATIONS IN URINE EXCRETION AND SODIUM AND POTASSIUM EXCRETION IN HEALTHY AND TOXÆMIC WOMEN IN LATE PREGNANCY

BY

PAAVO PYSTYNEN AND PAAVO PANKAMAA

The diurnal variations in the volume and composition of urine in man have long been under investigation Generally in healthy subjects excretion is greatest during the daytime and lowest during the night (Martel *et al.* 1962, among others) This cycle is resistant to alteration, such as night work and day sleep (Sharp, 1962) It does not depend on the number of meals (Wesson and Lauler 1961) high or low salt intake working or continual resting The diurnal excretory cycle is not altered by fluid retention due to hypertonic salt infusions, or by water retention due to vasopressin, or by salt retention due to desoxycorticosteroids (Wesson and Lauler 1961) It is interesting to note that in normal healthy subjects the normal diurnal variation in sodium chloride excretion occurs even during severe salt retention (Wesson and Lauler) but in subjects with congestive heart failure or hepatic cirrhosis the cycle is reversed, salt retention being followed by greater excretion by night and smaller excretion by day (Baldwin *et al* 1950 Fejfar and Brod, 1950 Jones *et al* 1952)

Many authors are of the opinion that the total body sodium of toxæmic women is greater than that of women with normal pregnancies *i.e.* there is active retention of sodium (Strauss, 1939 Dieckmann *et al* 1951 1957 Dieckmann and Pottinger 1955, 1957) We investigated and compared the diurnal cycle of urine excretion and of sodium and potassium excretion in preg-

From the index variations it can be deduced that during the time interval 23.31 to 7.30 the total excretion of urine is smaller than average. Only one experimental result is exceptional and statistically the results is highly significant ($P < 0.001$). During the period 9.31 to 19.30 the volume of urine excreted is increased. The determinations from four patients are contrary but in spite of this the trend is statistically significant. The interval 11.31 to 13.30 as a part of a period of higher excretion, is exceptional, the mean being practically 100. In spite of this, the mean for this period cannot be stated to differ significantly from adjacent means. The index of urinary excretion in the remaining periods is not statistically significant.

In groups 1 to 3 a similarity of relationships between different periods is present, compared both with each other and with normal groups.

As a similarity in the variations of different time intervals is present in groups 1 to 3 and groups 4 and 5, the most useful information may be obtained if all the determinations are analysed together. Now it can be seen that in the period 11.31 to 13.30 the excretion is smaller than in the preceding period. The mean of the indices derived from all determinations are presented here as whole numbers.

Time	*Index*	*Time*	*Index*
7.31–9.30	91	19.31–21.30	108
9.31–11.30	121	21.31–23.30	109
11.31–13.30	98	23.31–1.30	86
13.31–15.30	112	1.31–3.30	71
15.31–17.30	123	3.31–5.30	71
17.31–19.30	137	5.31–7.30	74

Excretion of potassium

Excretion of potassium largely follows the pattern of total urine excretion. There is a difference after 17.30, when the excretion is somewhat reduced, lasting until 1.30. The excretion is relatively higher during meals and between them. The indices of potassium excretion in all cases were as follows

Group 1	Hypertension of pregnancy	5 patients
Group 2	Mild pre-eclampsia	11 patients
Group 3	Severe pre-eclampsia	4 patients
Group 4.	Normal pregnancy	29 patients
Group 5.	Œdema of pregnancy	6 patients

Results and Statistical Analysis

The diurnal excretion of every patient was first determined. On the basis of this the average excretion during the two hour periods was then determined Each determination of the two hourly period was calculated as a percentage of the average and thus an index for each period has been achieved. In most cases the two hourly periods finished at 7 30 9 30, 11 30 and so on. In some of the cases the periods ended at some other time and in order to compare all these determinations two different interpolation methods were considered.

Firstly the formation of the index for each two hour period can be taken as corresponding to the average formation in the main group during the same time interval. On the basis of index alterations already determined in the main group for the same time interval it is possible to make time corrections for the smaller non-conforming group The advantage of this method seemed to be its ability to retain possible characteristic variations for the short (two hour) periods. Linear interpolation was considered as an alternative This method evens out the differences of sequential values but maintains the characteristics of the individual better During the subsequent analysis of the results the first method seemed to be misleading and for this reason all interpolations have been made using the linear method.

The mean of the indices was then determined for each patient at each time interval. The statistical analysis was begun with the normal group

Excretion of urine

In Groups 4 and 5 the mean value of the indices was very similar and therefore they have been combined into one group

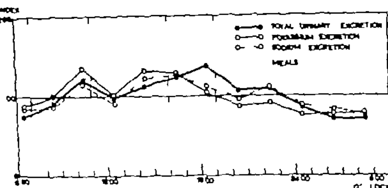


Fig. 1. The excretion of urine, potassium and sodium in all groups of patients.

mirrors the known diurnal cycle of normal, non-pregnant subjects. Conversely, it is the opposite of patients with such pathological conditions as congestive heart failure, hepatic cirrhosis or nephritis where the ratio is reversed, being smaller by day and greater by night. (As can be seen in Table I, reproduced from J. Clin. Invest., 31: 328, 1952.) It is also notable that the variations in sodium and potassium excretion are similar, waxing and waning with the cyclical variation in the quantity of urine excreted. If the results of this investigation can be generalised, it means that if the excretion of urine is small in women in late normal and toxaemic pregnancy, the excretion of electrolytes is also small, and vice versa.

SUMMARY

The authors have investigated the diurnal cycle of urine excretion, and the excretion of potassium and sodium of women at rest in the last month of pregnancy.

The series consisted of twenty-nine women with normal pregnancies, six with oedema, five with hypertension of pregnancy, eleven with mild pre-eclampsia and four with severe pre-eclampsia.

The investigation revealed that the rhythm of excretion when measured at two hourly intervals was similar in the different groups studied.

Time	*Index*	*Time*	*Index*
7 31– 9 30	100	19 31–21 30	88
9 31–11 30	135	21 31–23 30	91
11 31–13 30	102	23 31– 1 30	76
13 31–15 30	132	1 31– 3 30	76
15 31–17 30	130	3 31– 5 30	79
17 31–19.30	104	5 31– 7 30	88

Excretion of sodium

Excretion of sodium does not differ significantly from variations of the total urine and potassium excretions and closely follows potassium excretion. It does not, however decrease as much as potassium after 17 30 The indices of sodium excretion in all patients are as follows

Time	*Index*	*Time*	*Index*
7 31– 9 30	87	19 31–21 30	96
9 31–11 30	116	21 31–23 30	110
11 31–13 30	91	23 31– 1 30	89
13 31–15.30	123	1 31– 3 30	84
15 31–17 30	125	3 31– 5.30	77
17 31–19 30	112	5.31– 7 30	84

Fig 1 shows the excretion of urine potassium and sodium in all groups of patients. Because there was no significant difference between them they were treated as a single entity

Analysis of the Results

There was no difference in the diurnal cycle of the excretion of urine potassium or sodium in the different groups studied. All the groups were therefore treated as a single entity On the basis of this investigation it appears that women in late pregnancy normal as well as toxaemic, excrete less urine potassium and sodium during the night than during the day This conclusion

The variations in excretion of potassium and sodium followed each other closely as well as being related to variation in the volume of urine excreted.

The excretion of urine and electrolytes was smaller by night than by day

Acknowledgements

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Table I. *Studies of Diurnal Variations of Renal Function in Certain Pathologic States. Reproduced from J. Clin. Invest. 31: 328, 1952*

...vestigator	Pathologic State		Renal Function Variation		
			Studied	Observed	Attributed to
...incke ( ) (1893)	Nephritis Congestive heart failure		Water excretion	Nocturia	----
...ilson ( ) (1889)	Nephritis Congestive heart failure		Water and solid excretion	Nocturia, incr solid excr	----
...hberg [18] (1939)	Nephritis Congestive heart failure		Water and electrolyte excr	Nocturia	Decr renal v... ous press I... cardiac output... pronepos.
...od [10] (1946)	Glomerulonephritis		Creatinine clear U/P ratios.	Nocturia, decr H O reabsorb.	---
	Congestive heart failure			Nocturia, incr GFR, decr H_2O reabsorb.	----
...ldwin [21] ...al (1950)	Congestive heart failure	Edematous	Inulin and creatinine clear U/P ratios excr Na.	Nocturia, incr GFR, incr Na excr	- --
		Edema free		Decr urine flow decr GFR, decr Na excr (at night)	Nocturia not r... lated to level GFR.
...od & ...ljfar [20] (1950)	Congestive heart failure		Inulin, PAH clear cardiac output.	Nocturia, incr GFR, incr ERPF decr FF No change in cardiac output	Incr of blood flow to kidney... with diminishe... activity
...lts [17] (1950)	Cirrhosis Congestive heart failure		Water and Cl excr	Nocturia, incr Cl excr	Semi-recumbent position at night
...ldman [16] (1951)	Congestive heart failure Glomerulonephritis Cirrhosis		Na, K, H O excr	Nocturia, incr Na excr No change K excr	Unknown humoral factor...

of the similar observation in post-partum blood (for references cf Albrechtsen, 1959) It has been indicated, among other things, that a local fibrinolytic process, restricted to the uterus, is of decisive importance during menstruation, while as yet nothing definite is known of a similar process following normal delivery

The object of the present investigation was to approach these aspects by a comparative study of a number of coagulation factors and fibrinolytic components in uterine and peripheral blood collected after delivery of the placenta during menstruation, and during the course of abnormal uterine bleeding.

Material and Methods

The analyses were performed on blood samples from a total of 65 women. The first group comprises 24 women who had normal deliveries 30–60 minutes before blood sampling. The pregnancies had been normal in all cases. Before the sampling, it was ensured that no major damage to the birth canal had been sustained.

The second group comprises 10 women with entirely normal menstrual periods occurring at the expected time The blood samples were obtained on the first or second day of bleeding.

The third group of 31 women had long-lasting, profuse, and quite irregular uterine bleeding. Samples were collected on the first day of the bleeding or if that was not possible, on the second or third day Coagulation and fibrinolysis studies on uterine blood and venous blood from 15 of these patients have been published previously (Albrechtsen and Skjodt, 1963)

The blood was obtained from an arm vein after light application of a tourniquet, using potassium ammonium oxalate (Wintrobe) or 3.8 % sodium citrate ratio 1 10 as anticoagulant. The samples were collected into ordinary glass tubes (11×100 mm) or into Owren's plastic thrombotest tubes At the same time blood was collected from the vagina by a vaginal speculum or by the application of an occlusive pessary The blood collected into the plastic tubes was immediately centrifuged at 1500 r p. m. for 5 minutes (platelet-rich plasma) and studied at once. The blood

Skjødt Preben, and Albrechtsen, Ole K. Acta obst et gynec scandinav 44 416, 965

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COAGULATION AND FIBRINOLYSIS IN UTERINE BLOOD

(Post partum during Menstruation and during Abnormal Uterine Bleeding)

BY

PREBEN SKJODT AND OLE K. ALBRECHTSEN[1]

Formerly it was the general opinion that the hæmostasis following normal delivery was due mainly to uterine tonus and that intrauterine coagulation was a morbid phenomenon which might involve a risk of thromboembolic complications (Stoeckel 1925) According to more recent investigations, however intrauterine coagulation appears to be a normal occurrence and an important factor in post partum hæmostasis (Greenberg, 1946 Bienlarz 1956 Schwenzer 1960) It has been demonstrated that the blood which comes from the uterus after delivery of the placenta is unable to clot (Greenberg, 1945) as the fibrinogen has been consumed by the intrauterine coagulation.

This non-coagulability of post partum blood has been compared with the corresponding phenomenon in menstrual blood (Greenberg, 1946) Thus the bleeding from the uterus after delivery of the placenta has been regarded as a first, acute menorrhagic menstrual period Although there is not yet complete agreement concerning the reason why menstrual blood cannot coagulate this phenomenon is far better elucidated than that

[1] The experience on the thromboplastin activation test was gained by O K.A. while staying as a Fullbright fellow at the James F Mitchell Foundation, Institute for Medical Research, Washington 15 D C., U.S.A. (T Astrup)

of the similar observation in post-partum blood (for references cf Albrechtsen, 1959) It has been indicated, among other things, that a local fibrinolytic process restricted to the uterus is of decisive importance during menstruation, while as yet nothing definite is known of a similar process following normal delivery

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collected into glass tubes was centrifuged at 3500 r p m. for 30 minutes (platelet-poor plasma) and the plasma was removed and stored at −20 C for later study

The following analytical methods were used, items 1 and 2 concerning platelet-rich plasma and items 3–12 frozen platelet poor plasma after thawing

1 Recalcification time in seconds in a mixture of 0 25 ml plasma 2 ml veronal buffer and 0 25 ml 0 025 M calcium chloride.

2 Thromboplastin activation test by the method of Astrup and Ollendorff (1961) *This investigation concerns the* formation of plasma thromboplastin and thrombin in a given *plasma following recalcification. The procedure is as follows* 0 25 ml 0 025 M calcium chloride is mixed with 2.0 ml veronal buffer and placed in a water bath at 37 C for 60 seconds. 0 25 ml platelet-rich plasma is then added. The amount of plasma thromboplastin and thrombin formed in this incubation mixture is then determined by measuring the clotting time when 0.1 ml of the mixture and 0.1 ml 0.0083 M calcium chloride are transferred at 1-minute intervals to a number of pilot tubes each containing 0.1 ml normal platelet poor test plasma with a recalcification time of more than 4 minutes In a normal subject these clotting times will gradually become shorter indicating increasing amounts of plasma thromboplastin and thrombin in the incubation mixture The result of this test is plotted on a graph in which the time in minutes is the abscissa and the logarithm of the clotting time in the individual tubes in seconds the ordinate. A normal curve is shown in Fig. 1 It may be characterized by the values tl tmax t14, Tmax and by the recalcification time in the incubation mixture as stated in the figure.

3 Partial thromboplastin time as described by Nye, Graham, and Brinkhous (1962) using a partial thromboplastin preparation, thrombofax (Ortho) This test gives an overall assessment of all components in the three phases of blood coagulation except for factor VII The result was estimated in seconds, as the mean of two determinations.

4. Thrombin time by determination of the clotting time in a

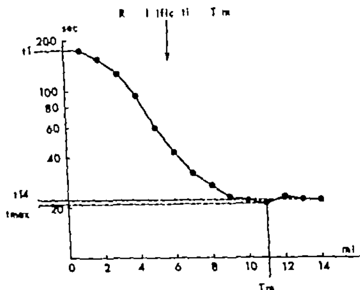


Fig Thromboplastin activation curve of peripheral blood from a normal, non-menstruating woman The curve is characterized by the following values
Recalcification time in the incubation mixture in seconds (marked by arrow)
t —coagulation time in the first tube, one minute after recalcification of the incubation mixture
3 T max—the number of minutes until the shortest clotting time is obtained.
4 t max the clotting time in seconds t T max
5 t 4—the clotting time in the fourteenth tube 14 minutes after recalcification of the incubation mixture

mixture of 0.1 ml plasma, 0.1 ml veronal buffer and 0.1 ml thrombin solution (20 NIH/ml dissolved in saline) at 37 C. The result was estimated in seconds as the mean of two determinations.

5.6 7 Prothrombin determination, determination of factor V and prothrombin-proconvertin as described previously (Albrechtsen and Skjodt, 1963)

8 Thromboplastin generation screening test according to Hicks and Pitney (1957) as previously described (Albrechtsen and Skjodt, 1963) The result of this test is plotted on a graph in which the time in minutes is the

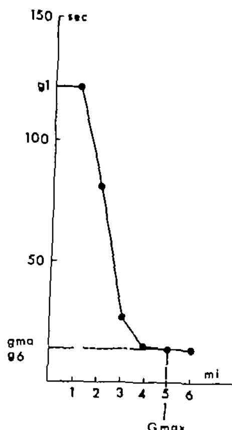


Fig. 2. Thromboplastin generation screening test on peripheral plasma from a normal, non-menstruating woman. The curve is characterized by the following values

1. g 1—clotting time in the first tube 1 minute after recalcification of the incubation mixture
2. G max—time in minutes until the shortest clotting time has been obtained.
3. g max—clotting time in seconds at G max.
4. g 6—clotting time in the sixth tube 6 minutes after recalcification of the incubation mixture

abscissa and the clotting time in seconds the ordinate (Fig 2) Like the thromboplastin activation test this investigation indicates the formation of plasma thromboplastin and thrombin in the plasma following recalcification, but an excess of platelet factor is present during the recalcification in the Hicks and Pitney test. The curve is characterized as shown in the figure by the values gl, gmax, g6 and Gmax.

9. Determination of the fibrinogen concentration was carried out on citrate plasma to which a 2 % solution of epsilon aminocaproic acid in veronal buffer in the ratio 0.2 to 1.2, had been added in order to counteract any fibrinogenolysis. The fibrinogen content was then determined as described previously (Albrechtsen and Skjødt, 1963) The result was stated in mg/100 ml as the mean of two determinations.
10. Plasminogen was determined as described by Alkjaersig, Fletcher and Sherry (1959) following activation of the plasma by streptokinase and determination of the ability of plasmin, if formed, to break down caseine while releasing tyrosine. The result was stated as the number of µg copper tyrosine released per ml activated plasma determined by spectrophotometry and stated as the mean of two determinations.
11. In a few cases the plasminogen content was also determined in the euglobulin fraction following activation with urokinase. 0.1 ml urokinase solution (Leo) (2 mg urokinase/ml veronal buffer) was added to 0.3 ml plasma precipitated at pH 5.9. After 30 minutes incubation at room temperature the plasmin, if formed, was estimated on heated fibrin plates (Lassen, 1952) and the result was stated in square mm as the mean of three determinations.
12. The fibrinolytic activity was determined on standard fibrin plates (Astrup and Müllertz, 1952) and heated fibrin plates (Lassen, 1952) using bovine fibrinogen 0.2 %. The heated plates were controlled daily by addition of the plasminogen activator urokinase in order to make sure that all the plasminogen had been destroyed by heating. The result was stated in square mm as the mean of three determinations. The activity was determined in untreated plasma as well as in the euglobulin following isoelectric precipitation at pH 5.9 (Astrup and Rasmussen, 1958)

The following determinations were also performed.

13. Hæmatocrit in Wintrobe's hæmatocrit tubes following centrifugation of blood stabilized by potassium ammonium oxalate (Wintrobe) for 30 minutes at 3500 r.p.m. The result was stated in %

14 Platelets counts after brief centrifugation of citrate stabilized blood at 1500 r p m. The counting was done in a Thoma's chamber with a depth of 0 05 mm, using a phase contrast microscope

15 Serum was prepared from unstabilized blood which after collection in glass tubes was left to stand for 2 hours at 37 C and centrifuged at 3500 r p m for 30 minutes. This serum was analysed immediately as stated for platelet-rich plasma and was also stored at −20 C for later analysis.

Results

The analyses of *peripheral plasma* collected from women one hour after normal delivery from menstruating women and from patients having meno-metrorrhagia are shown in Table I For comparison this table includes the results of a similar study of peripheral plasma from 24 normal non-menstruating women (Skjødt, in preparation) The values are broadly speaking identical from group to group However the content of prothrombin-proconvertin factor V fibrinogen, and plasminogen is increased after delivery At the same time the recalcification time is shortened in this group compared with the other three These differences are significant as far as the prothrombin proconvertin and fibrinogen concentrations are concerned.

Table I. *Peripheral plasma. Mean values for haematocrit platelets partial thromboplastin time recalcification time t 1 t max t 14 T max g 1 g max G max g 6 prothrombin-proconvertin concentration factor V concentration prothrombin time thrombin time fibrinogen concentration fibrinolytic activity of unprecipitated plasma on standard fibrin plates fibrinolytic activity of un precipitated plasma on heated fibrin plates fibrinolytic activity of precipitated plasma on standard fibrin plates fibrinolytic activity of precipitated plasma on heated fibrin plates plasminogen concentration determined following streptokinase activation and following urokinase activation (for details cf Methods) The standard deviation was calculated on the basis of the formula*

$$\sqrt{\frac{\sum d^2}{(n-1)\ n}}$$

where n is the number of determinations and d the deviation of the individual determination from the mean Number of determinations stated in ( )

The analyses of uterine blood from the three groups with bleeding also show distinct conformity in several respects (Table II). For instance, the platelet count, the concentration of prothrombin-proconvertin and of factor V were considerably reduced in all three groups compared with the corresponding values in peripheral plasma. However, the concentration of prothrombin-proconvertin in uterine blood after delivery is higher than in the other uterine blood samples, corresponding to the increased concentration of prothrombin-proconvertin in the peripheral plasma from these subjects. In all groups the blood from the uterus failed to clot after addition of thrombin, after recalcification, and after addition of thromboplastin and calcium, indicating the absence of fibrinogen.

The thromboplastin activation test and the thromboplastin generation screening test showed the same changes in uterine blood from all three groups. Thus, formation of thromboplastin or thrombin is absent or negligable following recalcification. Furthermore, the clotting time in the first pilot tube (t 1) was shorter in uterine blood than in the corresponding peripheral plasma.

Estimation of fibrinolysis in uterine blood, on the other hand, showed a considerable difference between the post partum group and the other two groups. Uterine blood following normal delivery has no fibrinolytic activity and contains the same amount of plasminogen as peripheral plasma from the same women (cf. Table I). In menstrual blood and in uterine blood from patients with meno-metrorrhagia there was considerable fibrinolytic activity and a reduced plasminogen concentration. There were appreciable individual variations from specimen to specimen, as is evident from the calculated standard deviation. There was fibrinolytic activity in 7 out of 8 samples, when unprecipitated menstrual blood was tested on standard fibrin plates and plasmin activity in 3 out of 8 samples tested on heated plates. This shows that a plasminogen activator, and occasionally plasmin, is present in menstrual blood. The uterine blood from patients with meno-metrorrhagia showed greater individual variations. Thus, there was no fibrinolytic activity in 8 out of 25 samples studied on standard fibrin plates. The average consumption of plasminogen

Table 1

	Peripheral Plasma			
	Normal Women Not Menstruating	Normal Women ½–1 Hour after Delivery	Normal Women Menstruating	Patients with Meno-Metrorrhagia
Hæmatocrit in %	43 ± 0 3 (15)	44 ± 0.8 (9)	44 ± 0.9 (10)	42 ± 1.0 (9)
Platelets x 1000/mml	366 ± 18 1 (20)	308 ± 26 4 (15)	329 ± 22 5 (10)	316 ± 14 9 (16)
Thromboplastin activation test.				
Recalcification time in sec.	367 ± 27 1 (20)	324 ± 9.7 (9)	376 ± 13.4 (10)	366 ± 10.6 (14)
t_1 in sec.	202 ± 7.0 (20)	198 ± 17 5 (9)	199 ± 12 3 (10)	215 ± 9 4 (13)
t_{max} in sec.	20 ± 0 3 (20)	21 ± 0 8 (9)	23 ± 0 8 (10)	22 ± 0.8 (14)
t_2 in sec.	21 ± 0 2 (20)	23 ± 0 7 (9)	24 ± 0 7 (10)	24 ± 0 8 (14)
T_{max} in min.	11 ± 0 3 (20)	11 ± 0.1 (9)	12 ± 0 1 (10)	11 ± 0 1 (14)
Thromboplastin generation screening test				
g_1 in sec.	121 ± 5.3 (24)	91 ± 10.0 (9)	132 ± 7 2 (10)	115 ± 6.5 (12)
g_{max} in sec.	11 ± 0.2 (24)	12 ± 0.5 (9)	13 ± 0.4 (10)	12 ± 0 6 (12)
g_0 in sec	12 ± 0 2 (24)	12 ± 0 6 (9)	14 ± 0.4 (10)	12 ± 0.6 (12)
G_{max} in min.	5 ± 0 2 (24)	4 ± 0.4 (9)	5 ± 0.3 (10)	5 ± 0.1 (12)
Partial Thromboplastin time in sec.	81 ± 1.0 (24)	78 ± 2 0 (9)	80 ± 2 4 (9)	74 ± 1 7 (10)
Prothrombin-Proconvertin in %	76 ± 2 7 (24)	145 ± 9.1 (22)	83 ± 3.2 (10)	83 ± 2 4 (30)
Factor V in %	91 ± 6 2 (24)	118 ± 12 0 (22)	88 ± 4 8 (10)	106 ± 6 3 (31)
Prothrombin time (Quick) in sec.	14 ± 0.2 (24)	14 ± 0 1 (3)	3 ± 0 3 (10)	16 ± 0 1 (30)

Thrombin time in sec	± 3 (24)	± 5 (5)	± 5 ( )	± 4 ( )
Fibrinogen in mg/ 00 ml	5 ± 9 8 (24)	443 ± 20 (22)	248 ± 7 3 ( )	8 ± 3 9 (27)
Fibrinolytic activ ty in plasma standard fibrin plates (mm)	(4)	(5)	( )	± 9 (30)
Fibrinolytic activity in plasma heated fibrin plates (mm²)	(4)	(5)	( )	0 (30)
Fibrinolytic activity in euglobulins standard f brin plates (mm)	6 ± (4)	6 ± 4 (9)	± 3-3 (9)	23 ± 8 6 (5)
Fibrinolytic activity in euglobulins heated fibrin plates (mm)	(24)	± 0 (9)	± 7 (9)	4 ± 2.2 (5)
Plasminogen in micrograms Cu. tyrosine/ml.	26 ± 6 5 (24)	48 ± 3 (9)	16 ± 5 6 (10)	1 9 ± 0. ( )
Plasminogen-urokinase activation of euglobulins heated fibrin plates (mm)	—	50 ± 9 2 (3)	—	—

was correspondingly less in this group than in the menstrual blood

The analyses of *peripheral serum* from women one hour after normal delivery from menstruating women, and from patients with meno-metrorrhagia showed conformable results. However there was a slight increased concentration of prothrombin proconvertin in the serum following delivery as compared with the other two groups

It is apparent from the tables that the values in peripheral serum (Table III) are in the same range as those in uterine blood. It was however an exception worth mentioning that menstrual blood and uterine blood from patients with meno-metrorrhagia showed fibrinolytic activity and a reduced content of plasminogen, while peripheral serum from these two groups did not show fibrinolytic activity and had a normal plasminogen concentration.

It has been demonstrated previously *in vitro* that weak solutions of tissue thromboplastin to which normal plasma has been added cause pronounced alterations of the thromboplastin activation curve manifesting themselves as considerable shortening of the recalcification time in the incubation mixture, a considerable shortening of the clotting times t 1 and tmax and a shortening of the time Tmax (Astrup and Ollendorff 1961) Thus this so-called three stage thromboplastin activation test may be used to decide whether a given sample contains free tissue thromboplastin in significant quantities. It has been demonstrated, furthermore, that addition of serum to normal plasma also gives rise to acceleration of the thromboplastin activation curve (Astrup and Ollendorff 1961) However these alterations differ from those mentioned above which were caused by tissue thromboplastin, as the recalcification time, the clotting time t 1 and the time Tmax, showed only a moderate shortening

Table IV sets out the results of such a three-stage thromboplastin activation test performed on peripheral plasma, vaginal plasma and peripheral serum from a limited number of subjects within each of the three groups having vaginal bleeding. The experiments were carrried out by replacing 0.05 ml of the peripheral plasma by 0.05 ml peripheral serum or 0.05 ml vaginal plasma in the incubation mixture. Thereupon the thrombo-

Table II. *Vaginal plasma. For details cf. Table I*

	Vaginal Plasma		
	Normal Women ½ Hour after Delivery	Normal Women Menstruating	Patients with Meno-Metrorrhagia
Hematocrit in %	5 ± 5 (7)	± 2.4 (5)	22 ± 1 1 (3)
Platelets x 000/mml	17 ± 8. (7)	3 ± 0 0 (6)	10.7 ± 4 0 ( )
Thromboplastin activation test			
Recalcification time in sec.	∞ (9)	∞ ( )	∞ (1)
t_1 in sec.	2 ± 5 5 (9)	25 ± 9 8 ( )	1 1 ± 12.0 (1)
t_{max} in sec.	9 ± 6 8 (9)	03 ± 5 5 (1)	82 ± 9 9 (1)
t_2 in sec.	95 ± 6 0 (9)	09 ± 6 4 (10)	83 ± 9.2 (1)
T_{max} in mm	± 1 (9)	6 ± 1 5 (1)	9 ± 1.2 (1)
Thromboplastin generation screening test			
g_1 in sec	88 – 5 4 (9)	70 ± 7 6 (7)	92 ± 7 6 (5)
g_{max} in sec	67 ± 4 4 (9)	54 ± 6 4 (7)	71 ± 2.2 (5)
g_2 in sec.	69 ± 5 (9)	59 ± 7 6 (7)	74 ± 1 3 (5)
G_{max} in mm	5 ± (9)	5 ± 0 3 (7)	5 ± 0. (5)
Partial Thromboplastin time in sec	———	———	———
Prothrombin-Proconvertin in %	4 – 4 3 (3)	9 ± 2 (7)	24 ± 2.8 (22)
Factor V in %	5 ± 5 (3)	2 ± (7)	3 ± 5 (22)
Prothrombin time (Quick) in sec	∞ (24)	∞ (6)	∞ (4)
Thrombin time in sec	∞ (8)	∞ (7)	∞ (4)
Fibrinogen in mg 00 ml	———	———	———
Fibrinolytic activity in plasma standard fibrin plates (mm)	(6)	243 ± 66 (8)	63 ± 42 0 (25)
Fibrinolytic activity in plasma heated fibrin plates (mm²)	(6)	5 ± 5 (8)	3 ± 3 8 (5)
Fibrinolytic activity in euglobulins standard fibrin plates (mm²)	0 (4)	———	170 ± 55 (8)
Fibrinolytic activity in euglobulins heated fibrin plates (mm)	(4)	———	20 ± 6 (7)
Plasminogen in micrograms Ca. tyrosine ml	4 4 (9)	16 ± 3 3 ( )	40 ± 15 ( )
Plasminogen-urokinase activation of euglobulins heated fibrin plates (mm²)	45 8 4 (4)	———	———

Table III. Peripheral serum. For details cf. Table I.

	Peripheral Serum		
	Normal Women [illegible]–1 Hour after Delivery	Normal Women Menstruating	Patients with Meno-Metrorrhagia
łematocrit in %	—	—	—
latelets x 1000/mml	—	—	—
hromboplastin activation test.			
Recalcification time in sec.	∞ (7)	∞ (7)	∞ (5)
t_1 in sec.	155 ± 1 4 (7)	147 ± 10.0 ( )	143 ± 11 5 (5)
t_{max} in sec.	130 ± 7 9 (7)	107 ± 4 1 (7)	114 ± 4 9 (5)
t_{14} in sec.	131 ± 9.0 (7)	110 ± 4 5 (7)	1 3 ± 6.3 (5)
T_{max} in min.	10 ± 1.2 (7)	12 ± 0 3 ( )	7 ± –0 (5)
hromboplastin generation screening test			
g_1 in sec.	06 ± 4.2 (7)	103 ± 4 0 (5)	105 ± 5 6 (4)
g_{max} in sec.	8 ± 3 8 (7)	91 ± 5.2 (5)	96 ± 5.4 (4)
g_4 in sec.	93 ± 5 0 (7)	98 ± 5.2 (5)	106 ± 7 3 (4)
G_{max} in min.	4 ± 0 5 ( )	4 ± 0.9 (5)	4 ± 0 5 (4)
artial Thromboplastin time in sec.	—	—	—
rothrombin-Proconvertin in %	46 ± 10.1 ( )	31 ± 2 7 (5)	23 ± 3 3 (4)
actor V in %	9 ± 3 5 (7)	3 ± 0 7 (5)	5 ± 1.4 (4)
othrombin time (Quick) in sec.	∞ (7)	∞ (5)	∞ (4)
hrombin time in sec.	∞ (7)	∞ (5)	∞ (4)
brinogen in mg/100 ml	—	—	—
brinolytic activity in plasma standard fibrin plates (mm²)	0 (5)	0 (5)	0 (3)
brinolytic activity in plasma heated fibrin plates (mm²)	0 (5)	0 ( )	0 (3)
brinolytic activity in euglobulins standard fibrin plates (mm²)	—	—	—
brinolytic activity in euglobulins heated fibrin plates (mm²)	—	—	—
asminogen in micrograms Cu. tyrosine/ml.	1-0 ± 14 7 (7)	1 8 ± 8 1 (6)	124 ± 8.3 (5)
asminogen-urokinase activation euglobulins heated fibrin plates (mm)	—	—	—

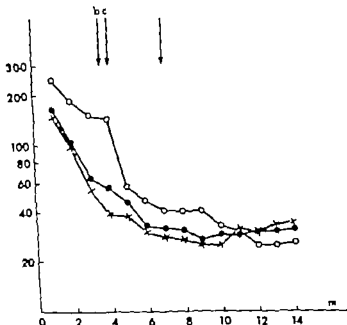


Fig. 3 Thromboplastin activation curves from a normal, menstruating woman (rec 1770/63–64)

a. ○ ——— ○ peripheral plasma
b. × ——— × peripheral plasma + vaginal plasma
c. ● ——— ● peripheral plasma + peripheral serum.

plastin activation test were carried out as previously described. The object of these experiments was to ascertain whether uterine blood from the three groups of women accelerated the thromboplastin activation curve as an indication of the presence of clot accelerating components. It is evident from the mean values recorded in the table that serum as well as vaginal plasma cause shortening of the recalcification time, shortening of the clotting time t 1 prolongation of the t 14 time and shortening of the time which elapses until maximum formation of thromboplastin and thrombin (T max.) has occurred. The vaginal plasma from all three groups showed a tendency to cause greater alterations than peripheral serum in the thromboplastin activation curve although this greater effect was not statistically significant. Fur-

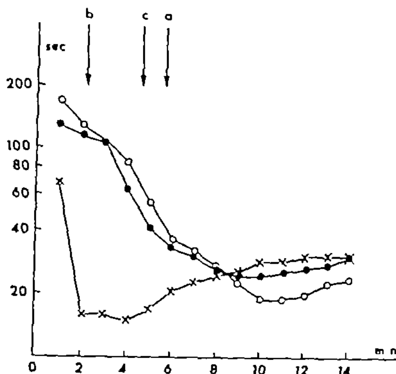


Fig. 4 Thromboplastin activation curves *from a normal, menstruating woman*
(rec. 1–5/63–64)

a. ○ ——— ○ peripheral plasma
b. × ——— × peripheral plasma + vaginal plasma
c. ● ——— ● peripheral plasma + peripheral serum.

ther analysis of the individual curves shows considerable variations from case to case within all three groups. Thus 6 out of 7 tested samples of menstrual blood caused the same alterations in the thromboplastin activation curve as peripheral serum from the same person (example in Fig 3) while one sample of menstrual blood exerted a considerably greater effect upon the thromboplastin activation in peripheral plasma than did peripheral serum (example in Fig. 4) Corresponding investigations of uterine blood from patients with meno-metrorrhagia revealed that only one sample affected the plasma thromboplastin activation curve in peripheral plasma in the same way as did the patient's peripheral serum while 4 samples caused greater activation than the patient's own peripheral serum. Lastly investigation of uterine

blood one hour post partum showed that 5 samples had the same effect as peripheral serum upon the thromboplastin activation curve while 2 samples had a distinctly more pronounced effect.

Discussion

The coagulation factors and fibrinolysis have been thoroughly studied in menstrual blood and in uterine blood from patients with meno-metrorrhagia (Beller 1957 Albrechtsen, 1959) The present investigation confirms the results of previous investigations. It reveals a consumption of coagulation components and increased fibrinolytic activity On the other hand, there have been only a few and isolated investigations into these factors in uterine blood during and after normal delivery A few studies have shown that while the blood coming from the uterus during delivery of the placenta clots very rapidly the blood which comes from the uterus during the early hours after the delivery of the placenta does not clot owing to total lack of fibrinogen (Greenberg, 1946 Bieniarz, 1956 Schwenzer 1960) This lack of fibrinogen is explained by the presumption that during delivery of the placenta intrauterine coagulation takes place because of the release of tissue thromboplastin from the placenta. This presumption has later been confirmed by the finding that during normal delivery there is a consumption of placental tissue thromboplastin. It has been demonstrated, for instance, that the content of tissue thromboplastin in placentae analysed after spontaneous delivery is less than the content in placental tissue analysed following manual removal during Caesarean section (Puder 1957 Schwenzer 1960)

The present investigation has confirmed that intrauterine coagulation takes place after delivery In the course of this process, a number of coagulation factors including fibrinogen, are consumed. The results of the studies on uterine blood were in keeping with similar studies on the serum prepared from the patients own peripheral blood following spontaneous coagulation. It has previously been discussed whether this intrauterine coagulation is followed by a fibrinolytic process (Stamm, 1962) Our studies do not indicate that this is so. In the first place neither plasminogen

Table IV *Mean values of the thromboplastin activation test on peripheral "plasma" from women 30–60 minutes after normal delivery from normally deviation calculated as stated in Table I Number of individual determinations*

	Normal Women / — Hour after Delivery			Normal Women
	Peripheral Plasma (25 cc)	Peripheral Plasma (20 cc) + Vaginal Plasma (05 cc)	Peripheral Plasma (20 cc) + Peripheral Serum (05 cc)	Peripheral Plasma (25 cc)
cification time sec.	324 ± 9.7 (9)	109 ± 8 6 (9)	39 ± 7 ( )	3 6 ± 13 4 (1)
sec.	198 ± 17 5 (9)	132 ± 7 3 (9)	151 ± 10 6 (7)	109 ± 1 3 (10)
in sec.	21 ± 0 8 (9)	0 ± 0 8 (9)	21 ± 0 6 (7)	23 ± 0 8 (1)
sec.	3 ± 0 7 (9)	0 ± 0 5 (9)	9 ± 0.5 ( )	24 ± 0 (1)
in min.	11 ± 0 1 (9)	8 ± 0 1 (9)	8 ± 0.6 (7)	12 ± 0.1 (1)

activators nor plasmin could be demonstrated in the uterine blood. In the second place the content of plasminogen was in the same range as in the peripheral plasma which is evidence against an activation process.

Thus, the non-coagulability of post-partum blood cannot be explained in the same way as the non-coagulability of menstrual blood. While the coagulation factors assessed in the present study show in principle the same changes in the two forms of uterine blood and can in both instances be interpreted as the consequences of intrauterine coagulation this coagulation process is followed during menstruation by a fibrinolytic process which redissolves the fibrin formed, while such a process does not occur after delivery This observation is in keeping with the finding that the human endometrium especially during the secretory stage, contains a plasminogen activator which is released during the bleeding, while placental tissue and decidual tissue possess no fibrinolytic activity (Albrechtsen, 1959)

It must be assumed, therefore that the intrauterine clot formed following normal delivery is stable The fact that nevertheless, liquid blood oozes from the uterus during the first hours post

plasma, peripheral plasma + peripheral serum and peripheral plasma + vaginal
menstruating women and from patients with meno-metrorrhagia. Standard
in ( )

[illegible] the Menstrual Period		Patients with Meno-Metrorrhagia		
Peripheral Plasma ([illegible] cc) Vaginal Plasma ([illegible])	Peripheral Plasma (0 10 cc) Peripheral Serum (05 cc)	Peripheral Plasma (0 15 cc)	Peripheral Plasma (10 cc) + Vaginal Plasma (0 05 cc)	Peripheral Plasma (0 10 cc) + Peripheral Serum (0.05 cc)
257 ± 27 (1)	288 ± 9 (7)	376 ± (9)	203 ± 22 6 (9)	238 ± 22.4
47 ± 19 6 ( )	65 ± 4 2 (7)	225 ± 9 (9)	3 ± 11 8 (9)	57 ± 5.4
24 ± 4 0 ( )	3 ± 8 (7)	1 ± 8 (9)	16 ± 1 (9)	18 ± 1.2
35 ± 8 ( )	30 ± (7)	3 ± 8 (9)	3 ± 9 (9)	27 ± 5
7 ± 0 6 ( )	8 ± 6 (7)	± 0 (9)	6 ± 0 7 (9)	8 ± 0 9

partum is possibly due to the uterine contraction after delivery promoting the expression of serum from the formed clot.

Uterine blood from patients with meno-metrorrhagia shows mainly the same values as menstrual blood. There is, however a tendency for the fibrinolytic activity including the consumption of plasminogen, to be a less constant phenomenon in these blood samples. It is reasonable to relate this to the fact that uterine blood from such patients, unlike menstrual blood, usually contains large clots. Further studies are needed to elucidate this question.

Several authors believe that intrauterine coagulation, during menstruation as well as following normal delivery is caused by the release of tissue thromboplastin, either from the endometrium or from the placenta and decidua (Whitehouse, 1914 Puder 1957) There is no final proof of this assumption. It would be supported by the demonstration of such tissue thromboplastin in the uterine blood. The fact that menstrual blood is capable of shortening the clotting time of normal blood (cf references given by Albrechtsen, 1959) cannot be taken to indicate that menstrual blood contains tissue thromboplastin, as serum possesses

similar properties. In the present investigation, attempts were made to elucidate this question by means of a three-stage thromboplastin activation test. The results were not consistent as in the majority of the cases studied the uterine blood merely accelerated the thromboplastin activation curve in peripheral plasma to the same extent as did the patient's own serum, while only a small number of samples accelerated the thromboplastin activation curve more than did the patient's own serum—indicating the presence of a coagulation promoting factor possibly tissue thromboplastin.

It is impossible to decide with certainty the possible importance of the intrauterine coagulation process to hæmostasis following normal delivery. However, it seems reasonable to assume that the tonus of the uterine muscles is operative as well as the coagulation process (Greenberg, 1945 and 1946; Bieniarz, 1956; Schwenzer, 1960). An isolated failure of one of these two mechanisms may entail hæmorrhage, for hæmorrhage occurs in association with atony as well as with systemic afibrinogenæmia.

The fact that the serum oozing from the uterine cavity after a normal delivery may shorten the recalcification time in normal plasma and accelerate the formation of plasma thromboplastin may possibly contribute to the coagulation disturbances in premature separation of the placenta. This complication is accompanied by the formation of a retroplacental hæmatoma and according to the recent investigations serum from this hæmatoma may find its way to the circulating blood (Nilsen, 1963). This explains why, despite a great loss of intrauterine blood and considerable anæmia, these patients may not become shocked, as their blood volume is not greatly altered. If the re-infused serum can accelerate the coagulation process in the circulating blood, there is a possibility of intravascular coagulation. This phenomenon, combined with a possible infusion of tissue thromboplastin from the placenta and decidua (Schneider, 1952) and with a consumption of fibrinogen in the retroplacental hæmatoma (Nilsen, 1963), may contribute to the occurrence of hypo- or afibrinogenæmia which is characteristic of some of these cases. In this connection, it is of interest that in a series of in

vestigations Wessler (1955) demonstrated that intravenous injection of serum may give rise to intravascular coagulation when venous congestion is present. Further analyses of this aspect have been planned.

SUMMARY

Investigations of the coagulation factors and fibrinolysis in uterine blood from normal women 1/2–1 hour after delivery from normally menstruating women and from patients with meno-metrorrhagia were compared with a corresponding analysis of the peripheral blood of the same subjects. This comparative investigation gave the following results

1. After a normal delivery intrauterine coagulation takes place. This process is not followed by an activation of the fibrinolytic system. The significance of this process to post-partum hæmostasis is discussed.
2. During normal menstruation also the coagulation studies indicate the occurrence of intrauterine coagulation. However this coagulation is followed by a fibrinolytic process with consumption of plasminogen.
3. In general the findings during meno-metrorrhagia are similar to the findings during normal menstruation. However fibrinolytic activity appears to be less constant. Further investigations are needed for a final elucidation of this aspect.
4. Uterine blood from all 3 groups like serum, contains a coagulation-promoting component. In a few cases the coagulating-promoting properties of uterine blood are, however more marked than those of serum. It is discussed whether this can be due t the presence of tissue thromboplastin in uterine blood

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AMNIOTIC FLUID EMBOLISM

A Case Investigated by Coagulation and Fibrinolysis Studies

BY

PREBEN SKJODT

Amniotic fluid embolism is a life-threatening complication which may arise during delivery or within the early hours post partum. The clinical condition is characterized primarily by acute shock accompanied by severe dyspnœa and cyanosis. If the patient survives this primary phase of shock, a hæmorrhagic diathesis may develop secondarily in the form of profuse uterine bleeding despite a contracted uterus bleeding from points of venipuncture ecchymoses, petechiæ gastrointestinal hæmorrhages, and hæmaturia.

This syndrome is due to massive escape of amniotic fluid into the maternal circulation. Steiner and Lushbaugh (1941) were the first to demonstrate particulate matter from the amniotic fluid in the pulmonary arterioles and capillaries. More recently Brozman (1961) has reported a well substantiated case of amniotic fluid embolism with platelet-thrombi scattered throughout the pulmonary vessels, allegedly caused by the particulat matter of the amniotic fluid. The result is a more or less extensive vascular occlusion which, combined with a reflex contraction of the vessels and possibly aggravated by the release of serotonin from the platelet thrombi, greatly reduces the pulmonary blood flow. This causes pulmonary congestion, pulmonary œdema, and shock. Electrocardiographic changes indicating acute cor pulmonale have been demonstrated (Arnold, Gardner and Goodman, 1961 Nickerson, 1961) Furthermore

chest radiography has disclosed dilatation of the right heart and characteristic changes in the lung fields Arnold *et al.* (1961) for instance, found bilateral perihilar densities 6 hours after the onset of symptoms which gradually subsided in the course of 3 weeks.

In a number of cases hypo- or afibrinogenæmia of the circulat ing blood has been suggested as an explanation of the hæmorrhagic diathesis occurring in patients with amniotic fluid embolism The cause of the hypo- or afibrinogenæmia has not yet been definitely elucidated. The hypothesis has been advanced that activation of the coagulation process within the vessels entails a consumption of the fibrinogen in the blood. Others feel that, on the contrary the lack of fibrinogen is due to breakdown of the circulating fibrinogen due to an activation of the fibrinolytic enzyme system of the blood. A combination of these two theories has also been proposed. Lastly it may be mentioned that in a few cases of hæmorrhagic diathesis in patients with amniotic fluid embolism the fibrinogen content of the blood has been found to be normal. The failing coagulability in these cases is supposed to be due to the presence of a circulating anticoagulant allegedly of heparin nature (Schneider 1959)

In the case of amniotic fluid embolism to be reported below the typical primary phase of shock was followed by a secondary hæmorrhagic diathesis.

Case Report

Case rec. 2182/62–63. A 24-year-old gravida I, para I, with no history of major illnesses or family history of hæmorrhagic diathesis The patient had never perviously shown a tendency to abnormal bleeding Her last menstrual period was on May 9, 962, the expected date of confinement being the end of February 1963. There were no complications during pregnancy and regular antenatal care was conducted by her doctor and midwife. There had been no hypertension or proteinuria, and no tendency to œdema.

On Feb. 25, 963 she was admitted because of moderate fresh vaginal bleeding without uterine contractions or pains. Her general condition was unaffected. The foetus was found to be in the cephalic presentation Foetal heart sounds were regular 120/min. There was no tension or tenderness of the uterus. The bleeding soon subsided following rest in bed

On Feb. 28 at 4 p. m. labour started spontaneously There was no bleeding

The contractions soon increased in intensity and during this period the patient was restless and anxious. At 9 5 p.m. vaginal examination was performed. The head was transversely placed in the mid-pelvis and the orifice was not quite fully dilated. Puncture of a large, tense bag of membranes yielded a small amount of clear amniotic fluid. Thereafter the head descended to the pelvic floor

After the puncture of the membranes the patient became even more restless and anxious. At the same time she began to show faint cyanotic hue in the face without simultaneous dyspnoea. Oxygen was administered. When the foetal heart sounds became affected, it was decided to deliver the infant by Kjelland's forceps. Shortly before the induction of general anaesthesia the patient had severe vomiting

At 9 50 p. m. a live-born girl weighing 4060 g was delivered. The delivery was effected without complications The placenta was delivered manually It was fairly large with biplacenta measuring × cm. It was complete, without visible infarcts or signs of premature separation. Intrauterine palpation indicated that the cavity was empty and the uterus intact and well contracted after i administration of methergin 5 ml There was no cervical or vaginal laceration The total loss of blood was estimated as 500 ml. The B P was 00/60. There was faint facial cyanosis, but no respiratory embarrassment

At 10.45 p m acute deterioration of the patient condition occurred. She had just recovered from the anaesthesia and developed violent dyspnoea as well as intense generalized cyanosis She became disoriented. The blood pressure fell to 85/40 and the pulse felt soft and tremulous, 10/min. Auscultation of the lungs revealed crepitations and rhonchi on both sides. There was no vaginal bleeding, the uterus was well contracted, and there were no signs of visceral bleeding As there was suspicion of amniotic fluid embolism, venous blood was immediately drawn for coagulation study and immediately after that infusion of human serum was instituted Fifteen minutes later blood transfusion (No) was started Despite these measures the blood pressure dropped even lower The dyspnoea and cyanosis increased in severity Antistin (Ciba) 4 ml was administered i The uterus was still well contracted, and there was only negligible bleeding from the vagina.

At 5 p m the blood pressure had fallen to 50 mm systolic, and the infusion of another bottle of human serum was started. Shortly after oozing vaginal bleeding was observed The vaginal blood contained only few small clots The bleeding soon increased in intensity At the outset, the uterus was well contracted, but gradually its tonus decreased, and oxytocin was administered

At 45 p m the patient had lost total of 1000 ml blood after the delivery Another two bottles of blood (Nos 2 and 3) were infused, but the haemorrhage continued with th same intensity Now there was also bleeding from the points of venepuncture The dyspnoea and cyanosis had considerably diminished At the time the results of the coagulation analyses on the enous

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On Feb 28 at 4 p. m. labour started spontaneously There was no bleeding.



Fig. Chest radiography (4 3 963) Small bilateral, perihilar confluent densities and dilatation of the pulmonary arteries, mainly on the left.

There was no proteinuria The B P was 30/ 00 At the completion of the treatment the Hb had been 66 % and increased, in the course of the next 3 days, to 86 %. Chest radiography (on March 4, 963) showed no signs of pneumonia, but bilateral, small, perihilar confluent densities. Furthermore there was dilation of the arteries in the hilar regions, especially on the left. Follow-up X-rays on 3 63 showed that these changes had disappeared (Figs and 2)

Analytical Methods

Venous blood was collected into Owren's thrombotest plastic tubes containing 1/10 vol. sodium citrate 3.8 % as anticoagulant. The stabilized venous blood was centrifuged partly at 1500 rpm. for 5 minutes (platelet-rich plasma) and partly at 3500 rpm. for 30 minutes (platelet-poor plasma)

The platelet-rich plasma was immediately used for

blood samples drawn at 10.45 were available. Upon addition of thrombin to diluted plasma a small frail clot formed, indicating hypofibrinogenæmia. Further addition of bovine fibrinogen (0.4 %) resulted in a solid clot which started dissolving in 10 minutes and had disappeared after standing for 45 minutes at 37 C. The condition was now diagnosed as increased fibrinolysis and hypofibrinogenæmia, and it was decided to treat the patient with epsilon aminocaproic acid (EACA) and human fibrinogen.

About one hour later (on March 1 at 0 10 a.m.) the infusion of EACA (25 ml (0 1 g/ml) in 500 ml 5 % glucose) was started. At the same time, blood transfusion No 4 was instituted, and oxytocin, 4 I.U was administered. The hæmorrhage was profuse and the uterus atonic. Twenty minutes later infusion of human fibrinogen (6.0 g in 300 ml physiological saline) was started. After the first 50 ml of this solution had been infused, the dyspnœa and cyanosis increased and a transient visual impairment was apparent. These symptoms soon subsided after the rate of infusion was slowed down. At 0 30 a.m. another rapid analysis was carried out. Addition of thrombin to the plasma did not form any clot. After addition of bovine fibrinogen a solid clot formed, which at 37 C started dissolving in half an hour and had completely disappeared in 3 hours.

At 1 a.m. the hæmorrhage was decreasing, the dyspnœa had ceased and cyanosis had disappeared. The blood pressure was 85 mm systolic. At regular intervals blood samples were taken for measurement of fibrinogen content and fibrinolysis. On the basis of the results and the patient's clinical condition the treatment with EACA, human fibrinogen, and blood was continued.

At 2.20 a m. the hæmorrhage had considerably decreased. The B.P was 80 mm systolic. At this time upon addition of thrombin to plasma, a small clot appeared and there were no longer signs of increased fibrinolysis.

At 3 30 a.m. the hæmorrhage had ceased. The total loss of blood was 3300 ml. On addition of thrombin to plasma a solid clot was observed, still without signs of increased fibrinolysis. The treatment with EACA was stopped. A total of 2.5 g had been administered.

At 5.30 a.m. the infusion of human fibrinogen was discontinued, after a total of 12 g had been administered. The patient had, moreover received a total of 2500 ml blood and 1100 ml human serum. The blood pressure was 105/70 and the pulse rate 112/min. Catheterization yielded 60 ml clear urine. Yet another bottle of blood (No 6) was now administered. There was no further bleeding.

During the subsequent 3 days the temperature was 39 C This elevation of temperature could be explained by puerperal infection with tenderness of the uterus and foetid vaginal discharge. There was no evidence of hæmolytic transfusion reaction, no vaginal bleeding, and no respiratory embarrassment. As early as the first puerperal day the urinary output was ample (oo ml) and the specific gravity was normal (o g). Serum creatinine on 7 March was 1 1 mg/100 ml, and the creatinine clearance on 2 March was 79 ml/min.

EACA was supplied through the courtesy of A/S GEA, Copenhagen.

The platelet-poor plasma was stored at −20 C for later examination

Determination of fibrinogen concentration, prothrombin time (Quick) prothrombin-proconvertin (P P) factor V (proaccelerin) factor VIII (antihaemophilic globulin A) and factor IX (Christmas factor) and thromboplastin generation screening test by the method of Hicks and Pitney (1957) were carried out as described previously (Albrechtsen and Skjødt, 1962 Skjødt and Albrechtsen, 1965)

Determination of factor VII (proconvertin) was performed by the method of Koller Loeliger and Duckert (1951)

Thrombin time determination was performed by adding 0.1 ml thrombin ROCHE (10 NIH/ml) to 0.1 ml undiluted plasma + 0.1 ml veronal buffer pH 7.4 The clotting time is normally 13 seconds. Prolongation of the clotting time will occur in the event of a reduced plasma fibrinogen level but it may also be due to the presence of inhibitors (antithrombin) Antithrombin of heparin type may be neutralized by toluidine blue and may be demonstrated by repeating the thrombin time determination, 0.1 ml veronal buffer being replaced by 0.1 ml toluidine blue 25 mg/100 ml physiological saline Return of the thrombin time to normal indicates the presence of heparin.

Fibrinolytic activity in the plasma and isoelectrically precipitated plasma (euglobulins) and determination of the plasminogen content following urokinase activation of precipitated plasma were studied by the fibrin plate method as described previously (Skjødt and Albrechtsen, 1965)

Results

The results of the coagulation and fibrinolysis studies are recorded in Figs 3 and 4 which also set out the intensity and duration of the haemorrhage and the treatment with human serum, blood, epsilon-aminocaproic acid (EACA) and human fibrinogen.

Shortly after the patient became shocked (at 10.45 p. m.) the platelet count was 156,000/cu mm, and the concentrations of



Fig 2 Chest radiography (13. 11 1963) The perihilar densities have disappeared. Vascular pattern normal.

(1) Platelet count in a Thoma's counting chamber of a height of 0 05 mm using phase contrast microscopy
(2) Determination of fibrinogen and demonstration of any fibrinolytic activity (rapid analysis) To 0.2 ml plasma, diluted with 1 8 ml veronal buffer pH 7.4, 0 2 ml thrombin ROCHE (100 NIH/ml) was added. With plasma from a normal person this procedure results in a stable clot which shows no signs of dissolution after standing for 24 hours at 37 C In the event of absent or defective clot formation—as seen in hypo- or afibrinogenæmia—0.2 ml 0.4 % bovine fibrinogen is added to the above mixture A clot is then formed which is left to stand for observation for fibrinolysis.

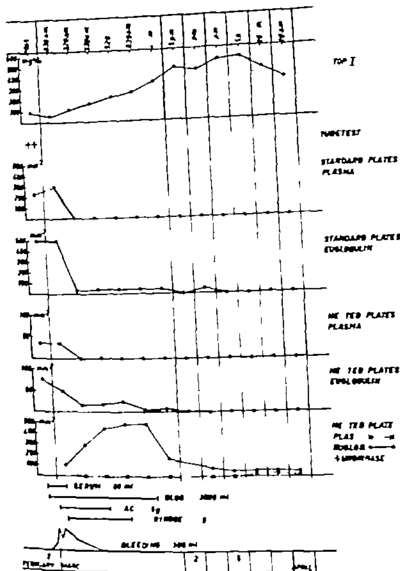


Fig. 4. Variations in the concentration of factor I (fibrinogen) and in fibrinolytic activity (elucidated by tube test and by the fibrin plate method: plasminogen activator + plasmin (standard fibrin plates), plasmin (heated fibrin plates) and plasminogen (heated plates following urokinase activation of the euglobulins)) in relation to the treatment and the extent of the bleeding

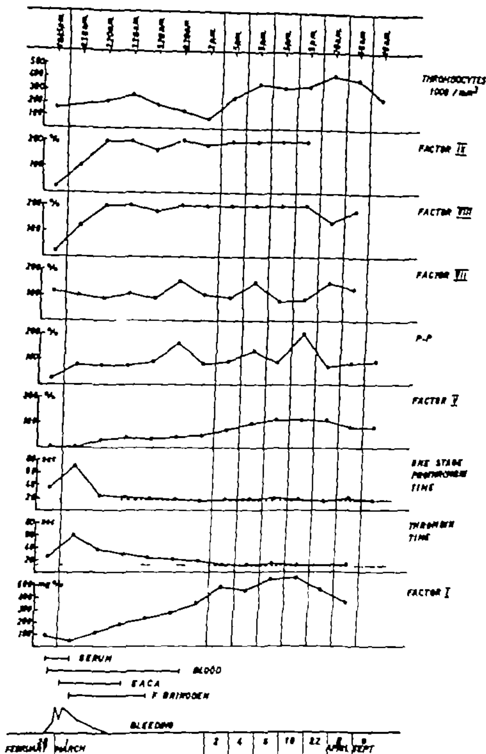


Fig 3 Variations in the concentration of the coagulation factors (platelet count, factor IX, factor VIII, factor VII, factor V prothrombin-proconvertin (P-P) prothrombin time thrombin time, and factor I (fibrinogen)) in relation to the treatment and the extent of the bleeding

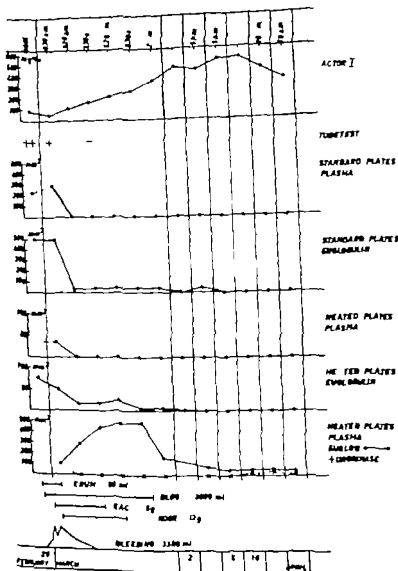


Fig 4 Variations in the concentration of *factor I (fibrinogen)* and in fibrinolytic activity (elucidated by tube test and by the fibrin plate method, plasminogen activator + plasmin (standard fibrin plates) plasmin (heated fibrin plates) and plasminogen (heated plates following urokinase activation of the euglobulins)) in relation to the treatment and the extent of the bleeding.

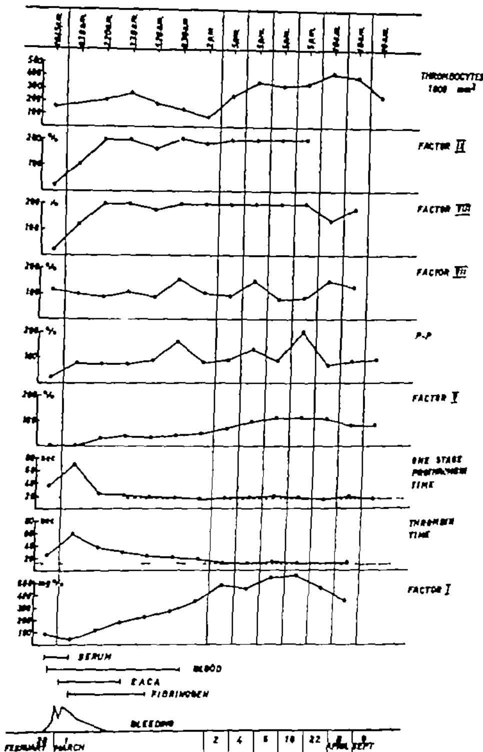


Fig. 3 Variations in the concentration of the coagulation factors (platelet count, factor IX, factor VIII factor VII, factor V prothrombin-proconvertin (P P) prothrombin time thrombin time and factor I (fibrinogen)) in relation to the treatment and the extent of the bleeding.

nogen concentration had risen to 110 mg/100 ml. The prothrombin time was almost normal, whereas the thrombin time was still greatly prolonged. Fibrinolytic activity was no longer demonstrable in the plasma by the rapid method or by the fibrin plate method. On the other hand, plasminogen activator and plasmin were still demonstrable in precipitated plasma, but only in limited quantities. There had been a marked increase in the plasminogen content.

At 3.30 a.m. the patient had received a further quantity of 500 ml blood 1/2 g EACA, and 3 g fibrinogen. The factor V concentration was still rising, and the fibrinogen concentration had reached 183 mg/100 ml. The thrombin time was still prolonged. There was as yet no fibrinolytic activity in the plasma, but a slight activity in precipitated plasma The content of plasminogen was still increasing rapidly At this juncture the bleeding stopped.

Through the subsequent 6 hours the treatment was completed by a further dose of 500 ml blood and 3 g human fibrinogen. The fibrinogen concentration rose evenly being 350 mg/100 ml at 2 00 p m. The thrombin time remained prolonged. There was still faint plasminogen activator and plasmin activity in precipitated plasma The content of plasminogen had appreciably decreased. Twenty-four hours later the coagulation status was normal.

The specimens which showed prolonged thrombin times were also submitted to thrombin time determination after addition of toluidine blue. The results showed that toluidine blue did not alter the prolonged thrombin times.

A thromboplastin generation screening test by the method of Hicks and Pitney (1957) was performed as described previously (Albrechtsen and Skjodt, 1962) This test reflects the formation of plasma thromboplastin and thrombin from the prestages factor VIII factor IX, factor XI (plasma thromboplastin antecedent) factor XII (Hageman factor) etc., calcium as well as platelet factors being present in excess. Fig. 5 gives the results. During the phase of acute shock—at 10.45 p.m.—there was a delayed and definitely reduced formation of plasma thromboplastin and thrombin—in accordance with the reduction

factor VIII (antihæmophilic globulin A) and factor IX (Christmas factor) were considerably below normal The content of factor V (proaccelerin) was extremely low The concentration of prothrombin-proconvertin (P P) was 23 % The prothrombin and thrombin times were prolonged. The fibrinogen concentration was found to be 90 mg/100 ml The concentration of factor VII (proconvertin) was 115 % and must be considered appreciably reduced as the content of factor VII in normal parturient women has been found to be much higher (Nielsen, 1963 Skjødt, in preparation)

Rapid analysis disclosed very pronounced fibrinolytic activity in the plasma Investigation by the fibrin plate method confirmed this finding In plasma diluted 1 10 with veronal buffer for instance an activity of 225 square mm was measured on standard fibrin plates and of 36 square mm on heated fibrin plates. A study of undiluted, isoelectrically precipitated plasma showed an activity of 449 square mm and 75 square mm respectively This indicates a high content of plasminogen activator and a considerable plasmin activity

After treatment with 1100 ml human serum, 1000 ml blood, and 1 g EACA (on March 1 at 0 30 a m) the concentration of factors VIII and IX considerably increased, and the prothrombin proconvertin value rose to 78 % On the other hand, the factor V concentration was still greatly reduced. The fibrinogen concentration had further dropped to 50 mg/100 ml, and the prothrombin as well as the thrombin time had become even more prolonged. Rapid analysis showed decreasing, but still pronounced fibrinolytic activity Investigation by the fibrin plate method still showed a high content of plasminogen activator and an appreciable plasmin activity in the plasma. Measurement of isoelectrically precipitated plasma still showed an unchanged high content of plasminogen activator and an intensive plasmin activity The plasminogen content was found to be reduced as compared with the findings in normal pregnant women.

At 2 20 a. m. the patient had received a total of 2000 ml blood, 2 g EACA and 6 g fibrinogen. The coagulation status was essentially improved, the platelet count and the factor V content showed a distinct tendency to rise. At the same time the fibri-

after the acute attack. The assumption that the condition was due to passage of amniotic fluid into the maternal circulation was further supported by the serological studies of Hostrup (1964) When the infant is a secretor large amounts of blood group substance corresponding to the infant s ABO group may be found in the amniotic fluid. During the phase of acute shock in the present case the concentration of A group substance in the maternal serum was considerably higher than later As the infant was group A and secretor the amniotic fluid must have contained appreciable quantities of group A substance. It is reasonable to assume therefore, that the high concentration of group A substance in the maternal serum was due to the passage of amniotic fluid into the maternal circulation.

The slight bleeding 4 days before delivery presumably issued from the marginal sinus of the placenta, as there were no signs of premature separation or deep insertion of the placenta. It is possible, therefore that the passage of amniotic fluid to the maternal circulation took place by way of the marginal sinus. The passage of the amniotic fluid was presumably favoured by strong uterine contractions and started after the membranes were punctured, since at that time the patient showed increasing unrest and anxiety and vomited. The delivery and the subsequent manual removal of the placenta may have given rise to further escape of amniotic fluid into the maternal circulation, with precipitation of acute, severe shock. The fact that shock did not manifest itself until nearly one hour after the delivery may be due to the anaesthesia.

Several theories have been advanced in explanation of the hypo- or afibrinogenaemia which may occur in patients with amniotic fluid embolism.

According to Weiner and Reid (1950) following passage into the maternal circulation the tissue thromboplastin of amniotic fluid, causes intravascular coagulation with consumption of the fibrinogen of the blood leading to deposition of fibrin in the pulmonary circulation. This view is not generally accepted. Animal experiments using intravenous injection of unfiltered amniotic fluid causes shock and defibrination, while injection of filtered amniotic fluid does not produce these symptoms (Stel-

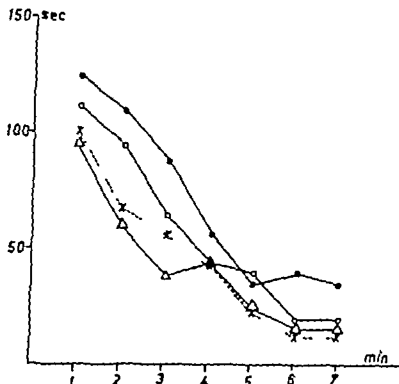


Fig. 5. Formation of plasma thromboplastin, and thrombin in the peripheral blood compared with normal control plasma.

● —— ● 10.45 p. m.

o —— o 2.20 a. m.

△ —— △ 3.30 a. m.

X - - - - X normal control plasma.

Abscissa Incubation period in minutes.

Ordinate Clotting time in seconds

which was found in the content of factors VIII and IX. At 2.20 a. m. there was marked improvement, and at 3.30 a. m. there was normal formation of plasma thromboplastin and thrombin.

Discussion

The diagnosis of amniotic fluid embolism in the present case was based upon the typical clinical course predominated by a primary shock phase and a secondary hæmorrhagic phase. It was supported by the X-ray demonstration of perihilar densities and excessive filling of the arteries in the lung fields as late as 4 days

had been started. It must be emphasized that in this case there were no signs of premature separation of the placenta. This must rule out any loss of fibrinogen in a retroplacental hæmatoma as a contributory cause of the hypofibrinogenæmia (Nilsen, 1963)

At the time of the first analysis (at 10.45 p m.) the low values found for factor IX (Christmas factor) (Donaldson, 1960 Lewis and Nour Eldin 1962) factor VIII (antihæmophilic globulin A) (Schneider 1959 Fletcher Alkjaersig, and Sherry 1961) factor V (proaccelerin) (Stefanini and Crosby 1950 Sherry Fletcher and Alkjaersig, 1959) prothrombin-proconvertin (P P) (Schneider 1959 Donaldson, 1960) and of fibrinogen may have been a consequence of an activation of coagulation intravascularly or of the highly increased fibrinolytic activity Something quite different applies to the considerable thrombocytopenia and reduction in the concentration of factor VII (proconvertin) Even greatly increased fibrinolysis does not induce a reduction in the number of circulating platelets (Koller 1961) or a reduction in the content of factor VII as this factor is very stable against plasmin (Brakman van Creveld, Engelsman, vant Laar Mechtar and Veder 1959) On the other hand, the thrombocytopenia and the reduced concentration of factor VII may have been caused by intravascular coagulation.

The platelet count is decreased as a consequence of an activation of the external coagulation system caused by tissue thromboplastin, as well as an activation of the internal coagulation system, involving formation of plasma thromboplastin (Koller 1961) It is generally accepted that factor VII is necessary for coagulation when the latter is activated by tissue thromboplastin (Owren and Bjerkelund 1949) On the other hand, factor VII is not needed for activation of the internal coagulation system, as normal formation of plasma thromboplastin has been observed in cases with isolated lack of factor VII (Ackroyd, 1955 Hicks 1955)

Investigations into the content of factor VII in the peripheral and uterine blood of normal parturient women have previously been carried out by the present analytical method (Skjodt, in preparation) One hour after delivery 11 women showed in the peripheral blood a concentration of 280 %±65 o The uterine

ner Lushbaugh and Frank 1942 Koutsky Bednar and Dejmal 1961) This indicates that tissue thromboplastin can hardly be the precipitating factor as the content of tissue thromboplastin is not altered by filtration. The provocative factor appears to be connected with the particulate matter of the amniotic fluid

McKay who also believes that the afibrinogenæmia is a consequence of intravascular coagulation thinks that corpuscular elements in the amniotic fluid give rise to the formation of platelet thrombi in the *pulmonary arterioles and capillaries. Breakdown* of platelets in these thrombi results in the release of platelet factor *III* which causes the formation of plasma thromboplastin. This again results in the conversion of prothrombin into thrombin which changes the fibrinogen of the blood into fibrin (Beller Douglas Debrowner and Robinson, 1963) In animal experiments such platelet thrombi in the pulmonary circulation have been demonstrated following intravenous injection of unfiltered amniotic fluid, and at the same time a pronounced decrease in the number of circulating platelets has been observed (Steiner Lushbaugh, and Frank 1942 Slunsky 1962) In one human case of amniotic fluid embolism platelet thrombi have been demonstrated in the *pulmonary vessels* (*Brozman*, 1961)

As already mentioned increased fibrinolytic activity is frequently present. It is possible that in some still unknown way the intravascular coagulation may activate the fibrinolytic enzyme system. It can by no means be ruled out however that the activation of the fibrinolytic system is the central factor so that the plasmin thus formed *gives rise to the coagulation defect* (Horowitz, Des Prez, and Hook 1962 Beller Douglas, Debrowner and Robinson 1963)

In a few cases of hæmorrhagic diathesis it has been assumed that a circulating anticoagulant of heparin nature was responsible (Schneider 1959)

In the present case of amniotic fluid embolism there were extremely favourable *conditions for investigating the* coagulation mechanism. The coagulation status was established immediately after the onset of acute shock and before intravenous infusion

cluding fibrinogen and formed fibrin, the increased fibrinolytic activity may have aggravated the coagulation defect to such an extent that half an hour after the onset of shock a rapid increase in vaginal bleeding started.

As the brisk fibrinolytic activity was considered a contributory cause of the profuse haemorrhage treatment with EACA was instituted for the purpose of inhibiting fibrinolysis before starting infusion of human fibrinogen. The patient received a total of 25 g EACA and 12 g human fibrinogen. During the course of the treatment control analysis were performed.

At 0.30 a.m. a total of 1 g EACA had been administered. Measurement on standard fibrin plates revealed a distinctly less marked activity in untreated plasma than in isoelectrically precipitated plasma from which the fibrinolytic inhibitors and also EACA have been partially removed (Blix, 1962) This difference between the activity in untreated plasma and in precipitated plasma may be taken as an indication of a certain inhibition of the plasminogen activator As the fibrinolytic activity in untreated plasma was still markedly increased, however the inhibition was far from effective Similar investigations using heated fibrin plates showed no significant difference in the activity indicating a lack of inhibition of plasma activity The plasminogen content was found to be reduced compared with the values in normal pregnant women—in accordance with the high content of plasminogen activator (Skjødt and Albrechtsen, 1965)

At 2.20 a.m. a total of 2 g EACA and 6 g fibrinogen had been administered. Investigation of untreated plasma showed no fibrinolytic activity neither on standard fibrin plates nor on heated fibrin plates. However the fibrinolytic activity in the plasma had not ceased because of an inhibition of fibrinolysis caused by inhibitors, such as EACA, as corresponding studies on precipitated plasma showed only a modest activity suggestive of a small content of plasminogen activator and plasmin. This might indicate a spontaneous cessation of the fibrinolytic activity which is in keeping with the fact that fibrinolytic activity is often very short lived. The plasminogen content had increased considerably and remained at a very high level during the subsequent hours not showing a tendency to decrease until the fibrinogen therapy had

blood collected at the same time showed a concentration of 86·6 $\pm$9·2. Thus the concentration of factor VII is considerably reduced during the course of the intrauterine coagulation process which is assumed to be activated by tissue thromboplastin, which originates from the decidua as well as other sources. Even though factor VII is not needed for the activation of the internal coagulation system and even though factor VII is still present in normal serum, it has not been definitely demonstrated that the factor VII content also may be reduced in the course of coagulation involving the formation of plasma thromboplastin.

Thus the thrombocytopenia and the reduction in the concentration of factor VII as well as the decreasing quantities of the other coagulation factors might indicate that intravascular coagulation has taken place. However, it cannot be decided whether this activation of coagulation is caused by tissue thromboplastin in amniotic fluid or by platelet factor III released from platelet thrombi in the pulmonary circulation.

The first analysis (at 10.45 p.m.) showed an increased fibrinolytic activity and a high content of plasminogen activator. This corresponds closely to the findings in two published cases of amniotic fluid embolism (Albrechtsen, Storm, and Trolle, 1955; Beller, Douglas, Debrowner and Robinson, 1963). While it was assumed by Beller *et al.* (1963) that the increased fibrinolytic activity was the sole cause of the coagulation defect, it is reasonable to assume that in the present case the fibrinolytic activity was induced secondarily by the intravascular coagulation. It is a possibility that the fibrinolysis may have been elicited by the state of shock. Indeed, an appreciable increase in fibrinolytic activity has been observed in shock (Phillips and Skrodelis, 1958).

Although intravascular coagulation had caused the massive coagulation defect demonstrated in connection with the acute shock, there was negligible uterine bleeding and no signs of hæmorrhagic diathesis during the first half-hour after the patient became shocked. Presumably, hæmostasis was obtained because of an increased coagulation activity in the presence of a still adequate fibrinogen content. It may be assumed, therefore, that owing to further breakdown of several coagulation factors, in

cluding fibrinogen and formed fibrin, the increased fibrinolytic activity may have aggravated the coagulation defect to such an extent that half an hour after the onset of shock a rapid increase in vaginal bleeding started.

As the brisk fibrinolytic activity was considered a contributory cause of the profuse hæmorrhage treatment with EACA was instituted for the purpose of inhibiting fibrinolysis before starting infusion of human fibrinogen. The patient received a total of 25 g EACA and 12 g human fibrinogen. During the course of the treatment control analysis were performed.

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been discontinued. Very probably the high plasminogen content was due to a great extent to the administration of human fibrinogen which is contaminated to a varying degree with plasminogen

At 0 30 a. m the values representing coagulation factors IX, VIII and prothrombin proconvertin were rising The explanation may be that these factors were being administered by serum and blood. The unchanged low values of factor V and the decreasing concentration of fibrinogen are no doubt due to the still high fibrinolytic activity as these factors are particularly susceptible to plasmin At 2.20 a m. the concentration of factor V and of fibrinogen also showed a rising tendency in accordance with the fact that fibrinolytic activity was no longer present in the plasma However the increase in the fibrinogen concentration was small in relation to the amount of fibrinogen which had been administered. This is no doubt because when fibrinogen infusion was instituted there was still pronounced fibrinolytic activity in the plasma, which may have caused a breakdown of the fibrinogen.

Prolongation of the thrombin time may be due to a reduced fibrinogen content, but it may also have been caused by the presence of antithrombin. In the first 3 analyses in which the fibrinogen content was reduced the prolonged thrombin times may have been due to the hypofibrinogenæmia or to antithrombin activity In the subsequent analyses where the fibrinogen concentration was sufficient, the prolongation of the thrombin times indicated the presence of antithrombin in the plasma. This antithrombin was not of heparin nature as addition of toluidine blue failed to correct the thrombin times The antithrombin activity may have been a consequence of intravascular coagulation (Klein and Seegers, 1950) but the breakdown of fibrinogen may also have resulted in the formation of the split products fraction D and fraction E which possess antithrombin activity (Bang, Fletcher Alkjaersig, and Sherry 1962) However the antithrombin activity was so slight that it can hardly have played any role as a factor in the hæmorrhagic diathesis.

Although the course in the present case was favourable as the haemorrhage ceased and the patient's clinical condition became

generally satisfactory several objections may be raised against the treatment.

Presumably too much fluid was infused intravenously during the phase of acute shock, as there was a tendency to an exacerbation of the dyspnoea and cyanosis at the commencement of fibrinogen infusion. These symptoms were considerably mitigated when the rate of infusion was slowed down. Moreover infusion of a total of 1100 ml human serum must have caused a certain haemodilution which may have contributed to the further fall in the fibrinogen concentration demonstrated after the treatment had been instituted.

Finally it must be concluded that the dosage of EACA (2.5 g) was too small. The investigations revealed that the fibrinolytic activity in the plasma was not sufficiently inhibited at the commencement of fibrinogen infusion, so that part of the administered fibrinogen was presumably broken down. A more intensive treatment in the dosage of 6 g every 4 hours, as recommended by Nilsson (1961) would no doubt have resulted in a more effective inhibition of the fibrinolytic activity Possibly a massive inhibition of fibrinolysis might have rendered the administration of human fibrinogen superfluous. This is of great interest in view of the risk of hepatitis resulting from administration of human fibrinogen (Cronberg, Belfrage, and Nilsson, 1963)

SUMMARY

In the present case of amniotic fluid embolism both mother and infant survived The clinical course was classical, characterized by a primary phase of shock and a secondary phase of haemorrhage The diagnosis was supported by radiological demonstration of characteristic changes in the lung fields. Further confirmation of the diagnosis was afforded by the results of serological studies.

The coagulation defects during the primary phase of shock comprised hypofibrinogenaemia, a highly increased fibrinolytic activity thrombocytopenia, and a marked reduction in the content of several coagulation factors. At a later stage there was an antithrombin activity not due to the presence of heparin.

The investigations indicate that the massive clotting defect was due primarily to intravascular activation of the coagulation process consuming the clotting factors of the blood, including fibrinogen. The increased fibrinolytic activity presumably was secondary to the intravascular coagulation and, presumably by the breakdown of fibrinogen, fibrin, and other clotting factors, brought about a further aggravation of the coagulation status and thereby precipitated the secondary hæmorrhagic phase.

Therefore, treatment with epsilon-aminocaproic acid was instituted for the purpose of inhibiting fibrinolysis before starting infusion of human fibrinogen. Moreover human serum and several bottles of blood were administered.

Repeated clotting studies during treatment revealed a rapid improvement in the coagulation status simultaneously with a gradual decrease in the amount of bleeding.

The effect of the treatment is discussed.

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METHOHEXITAL ANÆSTHESIA IN CÆSAREAN SECTION

BY

PETER F S. LEE

The problem of providing suitable anæsthesia for Cæsarean section has been debated for many years Because of the danger of depression of the infant by general anæsthesia many obstetricians and anæsthiologists have advocated the use of regional anæsthesia at least until the baby has been delivered. Spinal and extradural anæsthesia have also many advocates, especially in the U.S.A. These latter techniques may however be accompanied by hypotension respiratory embarassment and other complications which may be troublesome and dangerous Further all these techniques are time consuming and may be technically difficult in an uncooperative patient

Modern general anæsthesia has been shown as capable of providing anæsthesia which is safe for the mother and child. Most workers in the field use a sequence similar to that described by the late R. J Hammer Hodges (1959) namely thiopentone succinylcholine nitrous oxide and oxygen with controlled respiration. Hodges demonstrated that there was less fœtal depression with this technique than when ether cyclopropane or trichlorethylene was used to maintain anæsthesia. Montgomery (1961) showed that this sequence also caused less fœtal depression than when anæsthesia was maintained with Halothane.

The use of ultrarapid barbiturates such as thiopentone in obstetrics has been hotly debated for many years. It was first thought that the placenta offered a barrier to thiopentone for a period of approximately 12 minues (Hellman *et al* 1944) and so the induction-delivery interval was made as short as possible. That this premise was false has been shown by many

workers, including Crawford (1956) and Flowers (1959) It is now generally accepted that thiopentone is detectable in significant foetal blood concentrations within approximately one minute, and that the concentration is at a maximum at about 2–3 minutes, after which it steadily decreases. Many authorities hold the opinion that the more recently introduced oxybarbiturate methohexital has a shorter duration of effect than thiopentone. The methohexital molecule differs from the other anaesthetic barbiturates in having no sulphur in the molecule and 2 positions of unsaturation in the 5-position radical. There are 2 asymmetric carbon atoms in the molecule and thus 4 isomers The mixture of all 4 produces a short acting, potent anaesthetic agent, which has a tendency to produce excessive skeletal muscle activity The 4 isomers can be resolved into 2 pairs α and β and only the α, which has minimal skeletal muscle activity is used in clinical anaesthesia. The pH of the solution is approximately 11.0.

It would thus seem logical to use this preparation to induce anaesthesia for Caesarean section on the ground that there might be less depression of the foetus. Many workers (e.g. Barron and Dundee, 1961) have found that the duration of action of methohexital is shorter than that of thiopentone but there have been other reports conflicting with this arguing that there is no statistical difference However it is believed by many workers, e.g. Dundee and Moore (1961) that there is less depression of the Cardiovascular System than with thiopentone which is another fact to be borne in mind when considering a technique for Caesarean section. It has also been thought that methohexital is more quickly broken down in the liver than thiopentone (Gibson et al. 1955) As a large proportion of the blood returning from the placenta via the umbilical vein passes through the liver it seems possible that the foetal blood levels could fall more rapidly

Material and Method

I ha e now used methohexital in 56 consecutive Caesarean sections in Köpings lasarett. The technique of anaesthesia was as follows

0 5 mg atropine only for premedication The patients were all induced in the 'head-up position, and preoxygenated for 1–2 minutes 10 % Inverdex *i v* Infusion begun and a sleep dose of methohexital injected fairly rapidly followed immediately by an adequate dose of succinylcholine (75–100 mg) The Sellick cricoid pressure manœuvre was also used. The patient was then intubated and respiration controlled with nitrous oxide and oxygen (6/3 l/minute) giving an inspired oxygen concentration of 33 % Intermittent succinylcholine was then given as required. Moya *et al* (1965) have shown that hyperventilation causes a slow onset of respiration in the infant and so an attempt was made to inflate the patient adequately but not to overventilate her When possible a Drager volumeter was used to control the minute volume. A record was made of the induction delivery interval and an Apgar count taken at 1 minute after delivery according to the revised method (Apgar 1958) As can be seen in Table I the average dose of methohexital was 1 23 mg/kg, with a range of 0 75–1 8 mg/kg

Complications

We experienced no serious falls of blood pressure in the 56 cases and the average pre-delivery fall was 4.7 mm Hg There were no deaths in the series and no serious anæsthetic complications in the mothers One patient, a diabetic, who received a total of 550 mg succinylcholine did not breathe immediately afterwards and was thought to have a dual block She responded to 10 mg of edrophonium and was then given atropine and neostigmine after which her respiration was normal.

Results

The results of the infants Apgar scores in the whole series are listed in Table II It can be seen that over 78 per cent of the 56 cases have an Apgar score of 8 or more and nearly all of these are due to a colour marking on the Apgar scale of 1 instead of 2. This is by far the least significant of the 5 modes of the Apgar system The mean Apgar score for the whole series was 8 5 with

Table I. *Dosage of Methohexital*

Dose mg/Kg	Number of patients
0.7–0 9	3
9–1	1
1 3	3
1.3–1 5	14
5– 7	3
7– 9	2
Total	56

Table II *All Caesarean Sections Apgar Scores of Infants*

Apgar	No of patients		%
4	3	5 4	5 4
5		3 6	
6	3	5 4	6 0
7	4	7 1	
8	6	7	
9	17	48	78 6
		9 6	
Total	56	oo	100

Table III *Elective Caesarean Sections—N Fortal Distress Apgar Scores of Inf nts*

Apgar	No of patients		
4		3 5	3 5
5			
6		6 9	3
7		3 5	
8		6 9	
9	4	48	86.2
	9	3	
Total	29	oo	oo

a standard deviation of 1 2 Although the series is very small, these figures compare favourably with other series reported in the literature Table III shows the results in elective Caesarean sections, excluding diabetic patients. Here we have 86.2 per cent

Table IV Caesarean Sections Associated with Foetal Distress (Incl. all Diabetics.) Apgar Scores of Infants

Apgar	No of patients		%
4	2	7·4	7·4
5	2	7·4	
6	1	3·7	22·3
7	3	11·2	
8	2	7·4	
9	15	55·5	70·3
10		7·4	
Total	7	100	100

of infants with an Apgar score of 8 or over. The one low score in this group was on a baby with the cord four times round the neck. The results for the non-elective Caesarean sections can be seen in Table IV. All were performed in various degrees of emergency. They also include 5 diabetic patients, 3 of whose infants had an Apgar score of 9 and the remaining 2 that of 6. Both the very low Apgar scores were explainable on obstetric grounds. There were no stillbirths or neonatal deaths. Four of the babies needed endotracheal intubation and controlled respiration. One premature baby weighing 2300 g, had shown foetal distress and a marginal placenta praevia had been found. This infant had a small area of atelectasis which resolved, and the baby is now well.

Discussion

Table V shows the induction-delivery interval, against the Apgar score and the numbers in each box show the number of cases. Our average induction delivery was 9.9 minutes with a range of 3–24 minutes. The optimal induction-delivery interval is surely a very interesting question. If the concentration of the barbiturate in the foetal circulation continues to decline steadily after the first 2–3 minutes the longer the induction-delivery interval the less should be the foetal depression from the barbiturate. However the chance of a normal Apgar score (between

Table V *Induction Delivery Interval Apgar Scor*

verage -9.0 Minutes
ange 3 24 Minutes

APGAR SCORE

INDUCTION DELIVERY INTERVAL IN MINUTES

	4	5	6	7	8	9	10	Total
4								1
4—6				1		4		7
6—8					1	5	3	9
8—10					1	6	1	10
10— 2	1				4	6	5	17
—14		1		1		2		4
4				2		3		8
Totals	3		3	4	6	27	1	56

Table VI *Induction-Delivery Interval*

	Under 10 Minutes (27 Cases)		Over 10 Minutes (29 Cases)		
APGAR SCORE under 4	5—7	8— 0	under 4	5—7	8— 0
ELECTIVE C S	2(8 %)	9(82)	(5 5)	(5 5 %)	6(89 %)
NON-ELECTIVE C S	(6 %)	5(94)	2(8)	5(46 %)	4(36)

8–10) would seem to decrease steadily after a certain time, about 7 minutes according to Hodges (1959) It is interesting to observe that with an induction-deli ery interval of less than 10 minutes, we have had no Apgar score under 6 The explanation given for the relative foetal depression occuring with prolonged anaesthesia is that it is due to the complicating obstetric factors, such as diabetes and prematurity together with the stress of the operation itself rather than to the anaesthesia. If this is so then it would show only in those cases where there existed such conditions, together with a long induction-delivery interval. In a purely elective Caesarean section with no foetal distress then the induction-delivery interval should not be important. We re-examined our figures taking 10 minutes as the arbitrary dividing

line below which there was no Apgar score under 6 In Table VI it can be seen that the elective Caesarean section infants did not vary very much in their Apgar results above and below 10 minutes induction delivery interval Eighty-two per cent of the under 10 minute interval group had an Apgar score of 8 or above compared with 89 per cent in the over 10 minute group

However when we come to the group of infants delivered by non-elective section which also includes diabetics, 94 per cent of the infants born after an interval of less than 10 minutes had an Apgar score of 8 or more. With an induction-delivery interval of 10 minutes or more the change is quite dramatic and only 36 per cent had a score of 8 or more while 46 per cent had a score between 5–7 and 10 per cent a score of 4 or under

This surely supports the hypothesis that complicating obstetric conditions are themselves probably the cause of this decline, rather than the anaesthesia *per se* In fact there is little else that could explain this situation The inspired oxygen concentration to the mother is more than 30 % and the 65–70 % nitrous oxide cannot cause hypoxia in the baby although intermittent inhalation of a high percentage of nitrous oxide has been shown by Rooth (1963) to cause a relatively raised p CO and more pronounced metabolic acidosis Neither can succinylcholine be incriminated as it has been demonstrated conclusively (Moya and Kvisselgaard, 1961) that it does not pass into the foetal circulation unless excessive amounts of the drug are used.

In the present state of our knowledge it would seem that the sequence of a rapidly acting barbiturate, followed by succinylcholine nitrous oxide and oxygen and controlled respiration provides safe and pleasant anaesthesia for the mother In an emergency situation it also provides the speediest means of delivering the baby

On balance there would seem to be adequate evidence to recommend using methohexital to induce anaesthesia, as the concentration in the infant s circulation could possibly decrease more rapidly with this than other barbiturates It might thus be possible to envisage an emergency situation where the baby could be delivered after a very short induction-delivery interval with a diminished risk of foetal depression.

Conclusions

In our present state of knowledge, general anaesthesia using this technique is to be recommended as the method of choice for providing anaesthesia in Caesarean section. It ensures safe and pleasant anaesthesia for the mother and ensures good oxygenation. It can also provide anaesthesia with the shortest possible delay and is of greatest value in an emergency situation. There is the very minimum of foetal depression. An attempt should be made to effect delivery within 10 minutes of beginning anaesthesia, especially in cases associated with foetal distress or prematurity

SUMMARY

Fifty-six patients undergoing Caesarean sections were anaesthetised with methohexital, succinylcholine, nitrous oxide and oxygen and the results are reported. A record was made of the Induction-Delivery Interval and the infants Apgar score at one minute after birth. There were no maternal or perinatal deaths. The average Apgar score for the whole series was 8 5. In the elective Caesarean sections 86 2 per cent of the infants had an Apgar score of 8 or over In the non-elective Caesarean sections (including Diabetics) it was shown that with an Induction-Delivery Interval longer than 10 minutes the percentage of infants with an Apgar score of 8 or over was only 36 per cent, compared with 94 per cent when the Induction-Delivery Interval was less than 10 minutes.

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TREATMENT OF MENORRHAGIA WITH EPSILON AMINOCAPROIC ACID

A Double Blind Investigation

BY

LENNART NILSSON AND GÖRAN RYBO

Up to the present available means for treating menorrhagia have been inadequate Uterus-contracting agents of the ergot type have usually no effect. Curettage reduces bleeding only for one or two menstrual periods and subsequently the menorrhagic type of bleeding generally returns. Hysterectomy may be a satisfactory procedure in the case of elderly women but it is inappropriate for young women who wish to retain fertility

In 1959 Okamoto described an amino acid epsilon-amino-caproic-acid (EACA) which is an efficient inhibitor of the activation of plasminogen to plasmin (fibrinolysin) and will control bleeding in such cases.

The preliminary results of Rybo (1964) indicate that in functional menorrhagia i e in menorrhagia without anatomical changes in the uterus such as fibroids or polyps there is an increased endometrial content of tissue activators for plasminogen. This may affect the coagulation and, hence increase the loss of blood Consequently in menorrhagia there is theoretical justification for the use of EACA which inhibits the activation of plasminogen I M Nilsson, Björkman and Andersson (1961) L Nilsson Rybo and Hallberg (1964) L. Nilsson (1964) and Gennser (1964) have shown that in menorrhagia reduction in the loss of blood can be obtained by the administration of EACA It is difficult to estimate the effect of treatment in women with menorrhagia solely by the patient s own estimate

of the amount of bleeding A method for measuring the loss of blood described by Hallberg and L. Nilsson (1964) enables a better evaluation of the loss According to Hallberg and L. Nilsson (1964) in normal women there is only slight variation in the loss of blood between different menstrual periods but it may be considerable in women with menorrhagia Hence a spontaneous decrease may simulate a therapeutic effect. Moreover, psychological factors may influence menstrual bleeding, and, consequently the prescription of any drug might result in a reduction in the loss of blood

In the present investigation of the therapeutic value of EACA a double-blind technique was applied using EACA and a placebo in order to eliminate the influence of psychological factors and of spontaneous variation in blood loss

Material and Methods

The series consists of 37 women between 17 and 50 years of age who were referred to the department on account of suspected menorrhagia They had complained to their physicians either of excessive bleeding or of symptoms of anæmia. In the anæmic women abnormally heavy menstruation was assumed to be the cause of the anæmia

A common gynæcological examination was performed at the first visit Later on hysterosalpingography was done in all but 7 cases (minor bleeder s) To prove that the bleedings were ovulatory a premenstrual curettage was performed, all but 4 women underwent such a curetting The examination revealed 5 cases of fibroids 3 of which were submucous

In all the women the loss of blood was measured during two consecutive menstrual periods During these periods each patient received a preparation marked A and another marked B Either A or B consisted of granules of EACA, (preparation Epsikapron supplied by AB Kabi Stockholm) or a placebo according to random distribution No person who received treatment and no one who made the measurements had access to the code Thus the investigation was performed according to the principles applied in double-blind testing

The doses prescribed were 6 measuring spoons daily admi nistered orally for the first three days of the period and then 4 3 2 and 1 measuring spoon respectively for the following 4 days. With the active substance this corresponds to 18 12 9 6 and 3 g. respectively The women were instructed to discontinue the medication as soon as menstruation had ceased. Deviations from the prescribed doses occurred. These were usually due to the side effects produced by the active substance 17 patients were given less EACA than placebo.

The loss of blood was determined by the method of Hallberg, and L. Nilsson (1964) In accordance with Hallberg, Hög dahl L. Nilsson and Rybo (1964) a loss of blood exceeding 60 ml was defined as menorrhagia

Results

In Table I some laboratory and clinical data for each patient are recorded. During periods when EACA was administered the mean blood loss in the entire series was significantly smaller than during periods when placebo was given. The average loss of blood per menstruation for periods of EACA treatment was approxi mately 52 ± 8 ml whereas for those of placebo treatment the mean loss was 127 ± 22 ml. (Table II) The difference is statis tically significant ($p < 0.001$)

There were no differences observed in the effect of EACA treatment between women with or without fibroids.

Out of the 37 patients shown in Table I 26 lost more than 60 ml when given the placebo thus fulfilling the criteria for the diagnosis of menorrhagia Their mean loss of blood when treated with EACA was 61 ± 10 ml, and when they received the placebo it was 164 ± 29 ml. Also here the difference is statistically significant ($p < 0.001$) These cases showed a reduction in blood loss exceeding 60 per cent. (Table II)

Eleven of the women did not fulfil the criteria for menorrhagia When treated with EACA their average loss of blood was 31 ± 6 ml and when given the placebo it was 39 ± 5 ml. This 20 per cent reduction in the loss of blood was not statistically significant (p 0.4–0.3) (Table II)

of the amount of bleeding. A method for measuring the loss of blood described by Hallberg and L. Nilsson (1964) enables a better evaluation of the loss According to Hallberg and L. Nilsson (1964) in normal women there is only slight variation in the loss of blood between different menstrual periods, but it may be considerable in women with menorrhagia Hence a spontaneous decrease may simulate a therapeutic effect. Moreover psychological factors may influence menstrual bleeding, and, consequently the prescription of any drug might result in a reduction in the loss of blood.

In the present investigation of the therapeutic value of EACA a double-blind technique was applied using EACA and a placebo in order to eliminate the influence of psychological factors and of spontaneous variation in blood loss.

Material and Methods

The series consists of 37 women between 17 and 50 years of age who were referred to the department on account of suspected menorrhagia. They had complained to their physicians either of excessive bleeding or of symptoms of anæmia. In the anæmic women abnormally heavy menstruation was assumed to be the cause of the anæmia

A common gynæcological examination was performed at the first visit Later on hysterosalpingography was done in all but 7 cases (minor bleeder s) To prove that the bleedings were ovulatory a premenstrual curettage was performed all but 4 women underwent such a curetting The examination revealed 5 cases of fibroids 3 of which were submucous.

In all the women the loss of blood was measured during two consecutive menstrual periods. During these periods each patient received a preparation marked A and another marked B Either A or B consisted of granules of EACA (preparation Epsikapron supplied by AB Kabi Stockholm) or a placebo according to random distribution. No person who received treatment and no one who made the measurements had access to the code Thus the investigation was performed according to the principles applied in double-blind testing

Table II.

Number of Patients	Treatment	Mean Blood Loss ml.	Standard Error ±	p	Reduction in Blood Loss (in per cent)
37	EACA	51 9	7 6	3 205 < 0 00	59
37 (entire series)	Placebo	26 9	22.1		
26	EACA	60 9	0.	3 424 < 0.00	62 9
26 (blood loss more than 60 ml)	Placebo	64 3	28.5		
1	EACA	30 7	6	.006 < .4-0 3	20.3
1 (blood loss less than 60 ml)	Placebo	38 5	4 6		

For 29 patients investigated the reduction in blood loss was between 50 per cent and 90 per cent. For 2 patients the reduction was less than 50 per cent and in 6 patients treated with EACA no decrease in the loss of blood occurred.

As can be seen from Fig 1 loss of blood occurs mainly during the first 3 days of menstruation It is during this period that a reduction in bleeding is of real importance With the stated dosage of EACA, there was considerable decrease in bleeding *during* this period

The side effects are shown in Table I In 13 cases there were no side effects in connection with either EACA or the placebo. Thirteen patients had side effects only when given EACA. The symptoms were stated to be mainly nausea or orthostatism Four patients had side effects only when they received the placebo medication and 7 displayed side effects with both EACA and the placebo

Discussion

The results show that EACA treatment produced a considerable decrease in the loss of blood in women suffering from

Table I.

	PLACEBO		EACA	
No	Blood Loss ml	Side Effects	Blood Loss ml	Side Effects
1	685		98	nausea
2	580		191	meteorism
3	228	fainting	50	
4	206		95	
5	199		46	
6	162	diarrhoea	127	diarrhoea
7	161		65	
8	153	nausea	205	nausea, orthostatism
9	144		41	
10	139		21	some nausea in the morning
11	136		62	
12	135	abdominal pains	42	
13	132		72	dizziness
14	125	nausea	22	nausea
15	120	slight orthostatism	129	
16	120	slight orthostatism	39	mild nausea
17	117		32	headache
18	116		23	
19	90		56	nausea
20	88	abdominal pains on first day	34	
21	84		14	fainting
22	79		17	
23	77		36	meteorism
24	70	abdominal pains	14	anxiety
25	64		30	
26	61		23	nausea during the first two days
27	58		13	fainting on first day
28	54		65	
29	53	vomiting on third day	27	dizziness, nausea, orthostatism
30	52		9	
31	45		18	nausea, orthostatism
32	44		17	
33	30		20	fainting, nausea
34	29		57	
35	24		22	nausea, fainting
36	22	headache abdominal pains	32	meteorism
37	13		58	

showed that when no treatment was given 5 patients bled more during these periods than was observed in connection with either the placebo or the EACA therapy It is difficult to determine whether in the cases mentioned, the placebo caused a decrease as a result of psychological factors.

Side effects such as nausea and orthostatism are of common occurrence in EACA medication. This is demonstrated by the fact that in 13 cases side effects were observed only during treatment with EACA. The side effects may prevent the administration of the full prescribed dosage and this may influence the reduction in the loss of blood. It is very rarely that treatment had to be discontinued on account of the side effects.

SUMMARY

The effect of EACA on patients with menorrhagia has been studied and the results have been compared with those obtained on administering a placebo. A double blind technique was used and the blood loss during menstruation was estimated objectively In 26 out of 37 patients the blood loss in the placebo cycles exceeded 60 ml. In the EACA cycles the blood loss in these patients was decreased by 50–90 per cent

Side effects appeared in 24 out of 37 patients In 13 only in connection with EACA treatment, in 4 only with placebo treatment and in 7 with both EACA and placebo treatment. The principle symptoms were nausea and/or orthostatism

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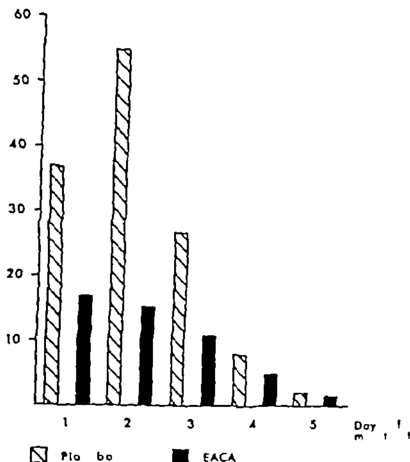


Fig. 1 Mean daily blood loss for 37 women with menorrhagia.

menorrhagia When using a special method for the objective determination of the menstrual blood loss and a double blind technique it was found that the effect of EACA could be due neither to spontaneous remission of menorrhagia nor to psychological factors. Obviously it must be the result of the antifibrinolytic effect of EACA

Six women lost more blood when they received EACA than when they were given the placebo but in 3 of these cases the difference was insignificant. In these 6 cases, however further determination of blood loss during one or more menstrual periods

Contrasting with all these investigations on animals are the few observations at least until recently on human immunization against sperm. The first cases were reported in 1954 by Wilson and Rümke. Wilson (1954) described two cases of men whose spermatozoa agglutinated spontaneously and whose blood serum and seminal plasma contained agglutinins against sperm and in 1956 he reported another case of the same kind. Wilson regarded this sperm agglutination due to antibodies as an hitherto unrecognized cause of sterility Two of his cases showed normospermia and one oligospermia Rümke (1954) reported two cases with sperm antibodies, both of which had oligospermia. Rümke and Hellinga (1959) investigated 2015 men in sterile marriages for antibodies against sperm. They found sperm antibodies in the serum of 67 i e in 3 per cent. The immunological investigations performed on this material made it appear probable that this state was due to an autoimmunization against sperm. Among the patients with sperm antibodies were 21 with azoospermia of these 16 had bilateral occlusion of the vas deferens. This was a significantly greater number than among the azoospermic patients without spermagglutinins. They concluded that spermatostasis resulting from occlusion of the vas deferens may lead to formation of sperm antibodies

Phadke and Padukone (1964) have confirmed this observation. These authors reported the presence of spermagglutinins in 8 out of 25 men in whom ligation of the vas had been performed and in 5 out of 25 men with obstructive azoospermia. They found sperm antibodies in 6 of 25 men after successful surgical treatment of obstructive azoospermia 3 of these 6 demonstrated normal fertility

Another cause of autoimmunization against sperm was reported by Cruickshank and Stuart Smith (1959) They found sperm-agglutinating antibodies in 2 out of 14 men with orchitis. The fact that infections of the genital organs such as epidydimitis and prostatic abscess can induce autoimmunization against sperm has been emphasized by Bandhauer (1963) He found sperm agglutinins in 9 out of 75 patients with such infections.

Nakabayashi, Tyler and Tyler (1961) in a survey of both fertile and infertile people found sperm-agglutinating anti-

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IMMUNOAGGLUTINATION OF SPERM IN CASES OF STERILITY

BY

BO FJÄLLBRANT

INTRODUCTION

Since the turn of the century sperm has been known to have antigenic properties. Numerous animal experiments relating to species and organ specificity have demonstrated that immuniza tion against sperm gives rise both to circulating antibodies which agglutinate and immobilize spermatozoa and to anaphylactic sen sitization

Experimental immunization of male animals with so-called adjuvantia has shown that immunization against sperm or tes ticular tissue can arrest spermatogenesis by destroying the spermatogenic cells. Some investigators have been able to produce this effect also with autologous material In some experiments on female animals immunization against sperm has induced a transient reduction of fertility This anti fertility effect has at tracted particular attention and given rise to great expectations about the possibility of reducing the overpopulation of the world by immunological means.

Comprehensive reviews of the animal experiments in this field during the first six decades have been given by Katsh (1959) and Tyler (1961) Of particular interest among the investiga tions during recent years are the transvaginal immunization against sperm performed by Behrman and Otani (1963) and the autoimmunization which Boughton and Spector (1963) produced by damaging one of the testes in situ

violent blows to the scrotum by footballs after puberty One man had a repair of an inguinal hernia before the age of one year and subsequently about 25 years of age had very transient swelling and tenderness of his contralateral testis. Otherwise none of the subjects gave any history of operation or other trauma to their genital organs or of genital infection In all cases the infertility was primary and none of the male partners had any extramarital children All of the 11 men were well and had normal sexual activity On clinical examination penis scrotal contents and prostate were normal with one exception a man who had a small spermatocele on each side.

In one case there was slight oligospermia with a sperm density of about 20 million per cc. In the other 10 cases semen examination gave normal quantitative and qualitative values.

Examination of the female partners, which in most cases included tubal insufflation hysterosalpingography and curettage did not reveal any cause for sterility Two of the wives had bicornuate uterus, but otherwise no significant cause of even relative infertility was found One of the wives had a pregnancy in a previous marriage A former wife of one of the patients in a previous infertile marriage became pregnant by her new husband after remarriage

In 4 cases homologous artificial insemination was carried out without success

The following is a typical case history

The patient was a 42 years-old man His previous marriage of several years duration was infertile and ended in divorce His former wife remarried and immediately became pregnant by her new husband. The patient remarried and the second marriage of years duration, was also infertile. In 958 the couple attended the clinic because of infertility The wife was normal on clinical examination, and hysterosalpingography endometrial biopsy tubal insufflation and basal temperature records also were normal. A semen examination gave the following values. volume 4.7 cc; sperm density 30 million per cc motility of the spermatozoa 1 hour after ejaculation, 75 per cent and normal abnormal spermatozoal heads, 5.5 per cent. In 959 cervical dilatation was performed and treatment with oestrogens was given As no conception had occurred by 960, a curettage was carried out Tubal insufflations in 960 and 96 gave normal kymographic tracings. In repeated postcoital tests during these years spermatozoa were found in the vagina in normal numbers but few if any spermatozoa were found in the

bodies in a small proportion of the infertile men However sperm from one donor used as antigen in the tests gave positive results considerably more often than the sperm from two other donors. A few women with sperm antibodies were found. These findings led Franklin and Dukes (1964) to make a more extensive investigation of the presence of spermagglutinins in women. Such antibodies were found in the serum of 15 of 19 patients with unexplained infertility

Positive results have been obtained much more frequently with the hæmagglutination technique than with the methods involving direct sperm agglutination Nakabayashi Tyler and Tyler (1961) found spermagglutinins in 18 per cent of the sterile individuals examined using hæmagglutination technique Southam (1963) using the same technique found agglutinins against seminal plasma in 12 per cent of males and 12 per cent of females examined. The most remarkable results of her investigation however were firstly the finding of agglutinins against seminal plasma in 35 of 51 pregnant women and secondly pregnancies occurring in some of the other patients with sperm antibodies.

In this paper some cases will be presented of men in infertile marriages who had sperm antibodies detected because of marked spontaneous agglutination of sperm and the significance of the antibodies with regard to sterility will be discussed

MATERIAL

The series comprises 11 men whose spermatozoa showed marked spontaneous agglutination and whose serum was shown to contain antibodies against sperm

Nine of the cases were found during routine examination of semen from 263 men who attended the Second Department of Obstetrics and Gynæcology in Göteborg because of infertility They comprise 3.4 per cent of the infertility cases of the clinic during the period of the investigation The other two cases had been sent to the clinic because of sperm agglutination observed by a practitioner whom they had attended because of infertility

The 11 men were 25–43 years old. Two of them had received

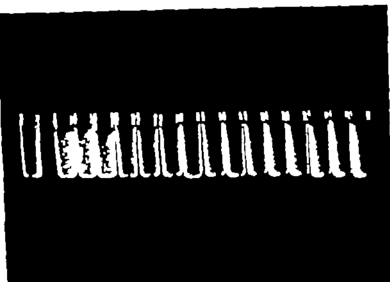


Fig Sperm agglutination test (Kibrick's method) T the left a negative control In this series agglutination can be observed until the eighth tube.

Test for sperm immobilizing antibodies

Those sera which were found to contain spermagglutinins were tested also for the presence of sperm-immobilizing antibodies Inactivated serum by itself had only a slight immobilizing effect. For a full immobilizing effect complement was needed. The following method was used

0.4 cc fresh semen (from the same donor as for the agglutinin titre determinations) and 0 2 cc fresh guinea pig serum of known complement activity (5 Lange Units) were added to 0.4 cc inactivated patient serum. After vigorous stirring to disperse the agglutinated spermatozoa at intervals drops of the mixture were transferred to a slide and the proportion of motile spermatozoa was estimated under the microscope

Postcoital test

The postcoital test was part of the routine infertility investigation Vaginal secretion taken from the posterior fornix, cervical

cervix, and not a single spermatozoon was ever found in the uterine cavity When the same result was obtained in 1962, a bacteriological examination of the cervical secretion was made. No infection of importance was revealed, but as a precaution the wife was given treatment with penicillin and oestrogens. After this treatment a failing sperm migration was still demonstrated by postcoital examination. Several homologous artificial inseminations were then made without conception ocurring. At a new semen examination in 1963 marked sperm agglutination was noticed. Serological tests demonstrated that the blood of the male partner contained antibodies against sperm.

METHODS

Semen examination

The semen samples were delivered to the laboratory of the clinic about 1–2 hours after ejaculation and were examined for colour consistency viscosity volume sperm density the percentage of motile spermatozoa and the quality of the motility the presence of leucocytes and other cells and the percentage of abnormal heads and their types. If agglutination of the spermatozoa had occurred the degree and type were assessed.

Test for agglutinins against sperm

When conspicuous agglutination of the spermatozoa was found at the semen examination the serum of the patient was tested for agglutinins against sperm The metod of Kibrick *et al.* (1952) was used

The patient s serum was inactivated by heating to 56 C for 20 minutes and was serially diluted twofold. Semen of good quality from the same donor was used for all the determinations a fresh ejaculate was diluted with Baker s solution (buffered glucose) to a sperm density of 40 million per cc The sperm solu tion was mixed with an equal volume of 10 per cent gelatine in Baker s solution and 0 3 cc of this mixture was added to 0 3 cc of the serum to be tested The resultant mixtures were transferred to a series of precipitation tubes (5 × 65 mm) and incubated at 37 C for 2 hours after which the presence of agglutination was assessed macroscopically (Fig 1) The highest dilution which gave quite obvious agglutination was accepted as the agglutinin titre



Fig. Macroscopic appearance of semen. Left tube—normal semen. Right tube— semen sample with immunoagglutination after sedimentation of the agglutinates

out quite distinctively against the almost clear supernatant (Fig. 2 right tube)

A drop of normal semen, placed on a slide and covered by a cover-glass, forms a thin even almost transparent layer which remains unchanged if the slide is kept for some hours in a moist chamber (Fig. 3 left field)

In cases of immunoagglutination large agglutinates are found under the same conditions. They could be seen within 1–2 hours after ejaculation (Fig. 3 right field)

secretion taken with forceps, and secretion from the uterine cavity aspirated with a cannula via the cervix were examined for the presence of spermatozoa and their motility The tests were made at the estimated ovulation time.

In vitro cervical mucus invasion test

In all cases where antibodies against sperm were found and in some other cases with reduced sperm migration at the postcoital test or with conspicuous sperm agglutination the cervical mucus invasion was studied *in vitro* For this purpose a drop of normal ovulatory cervical mucus was placed on a slide and covered with a cover-glass the corners of which were supported by small vaseline pillars In the narrow space between the slide and the cover-glass semen was allowed to flow in and surround the cervical mucus. The invasion of the spermatozoa into the mucus was then studied microscopically

RESULTS

Out of 263 patients 21 were found to have conspicuous sperm agglutination In these 21 cases the serum was tested for sperm-agglutinins. 9 sera were positive

In the 9 cases of immunoagglutination the spontaneous sperm agglutination was so conspicuous and massive that it could easily be distinguished from other types of agglutination both macro- and microscopically

Macroscopic picture

Normal semen retains its opalescence and even grey colour for many hours and the spermatozoa remain motile and dispersed throughout the ejaculate (Fig 2 left tube) A sediment may be formed if the semen contains many immotile spermatozoa or other cells but the supernatant never becomes clear

In the cases of immunoagglutination the agglutinates were conspicuous within one hour after ejaculation After 1–2 hours they settled at the bottom of the tube forming a sediment which stood

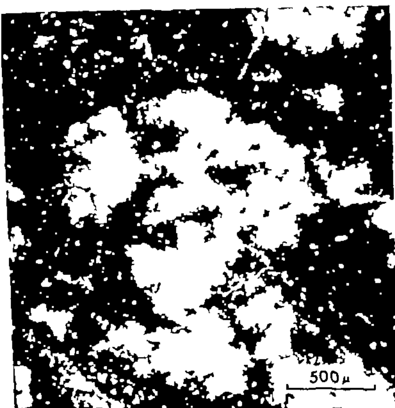


Fig. 4. Immunoagglutination of sperm.

The sperm immobilization test

The immobilizing effect of serum in the 11 cases of immunoagglutination compared with that of the serum from a puerperal patient, is seen in Fig. 6. In 8 cases immobilization was almost complete after 4 hours. In 2 cases it was considerably slower. In Table I the immobilization rate is that time within which 90 per cent of the spermatozoa had been immobilized.

The post coital test

Common to all cases of immunoagglutination was a reduced sperm migration. No spermatozoa were found in the uterine

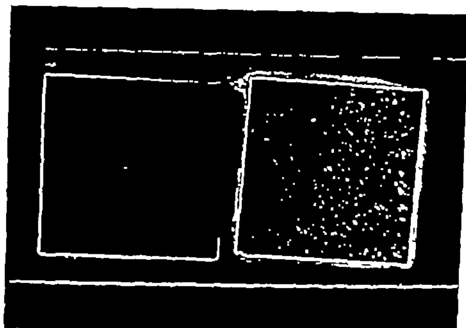


Fig. 3. A slide with normal semen to the left and semen with immunoagglutination to the right.

Microscopic picture

In cases of immunoagglutination the agglutination was quite conspicuous microscopically when the semen samples arrived at the laboratory 1–2 hours after ejaculation (Figs. 4 and 5). In one ejaculate that was examined immediately after ejaculation the first small agglutinates were observed after 10 minutes. The spermatozoa clumped tail to-tail and in one case also head to-head in conglomerates of gradually increasing size and density from which they appeared to be struggling to become free. In one case agglutination was almost complete but in the other cases some spermatozoa moved about between the agglutinates even after many hours. The agglutinates were partially dispersed by shaking but not even vigorous stirring would disperse them completely.

Agglutinin titre

The agglutinin titre varied between 1 : 32 and 1 : 16 384. The titres for each case are seen in Table I.

Table I.

No. of Case	Agglutinin Titre	Time Until 90% Immobilization
I	1 2048	hr 1 min.
II	28	4 hr
III	024	3 hr 0 min.
IV	8 92	hr
V	2048	hr 50 min.
VI	1 16384	hr 20 min.
VII	2048	3 hr 40 min.
VIII	5	3 hr 50 min.
IX	32	hr
X	024	6 hr
XI	64	24 hr

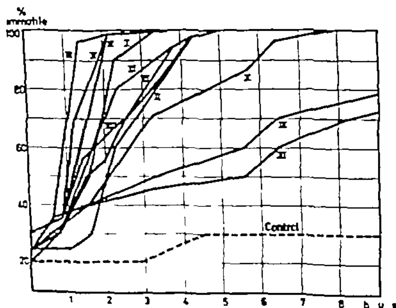


Fig. 6 Representation of the sperm immobilizing effect of serum in the cases of immunoagglutination

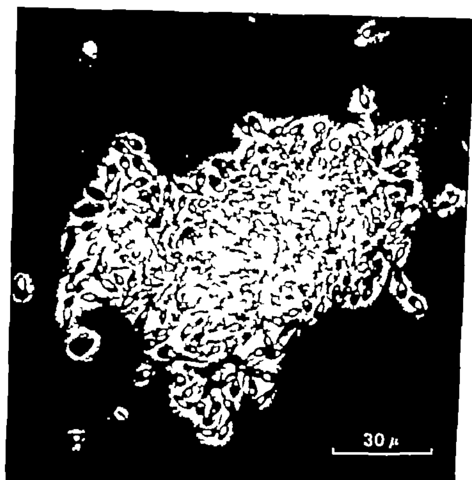


Fig. 5. Immunoagglutination of sperm.

cavity in any case In the majority of cases, despite a large number of spermatozoa in the vagina only a few spermatozoa could be discovered in the lower part of the cervix and none in the upper part. In case VI no spermatozoa could be seen even in the lower part of the cervix Only in cases IX and XI had a large number of spermatozoa invaded the cervix in case IX even the upper part of the cervix had been invaded The spermatozoa found in the cervix were immotile with a few exceptions in which the motility was very sluggish.

The in vitro cervical mucus invasion test

This test confirmed the reduced ability to invade cervical mucus

DISCUSSION AND CONCLUSIONS

The *method* of Kibrick for the detection of antibodies against human spermatozoa has been used by others e g Rümke and Hellinga (1959) for the investigation of autoimmunization against sperm. To us the method seems reliable in so far as we have had no difficulties in distinguishing positive tests from negative ones. The two-fold serial dilution however does not give a very distinct transition from one tube with agglutination to a neighbouring one without agglutination, and therefore the titre determination is somewhat subjective but within narrow limits (1–2 titre stages) Greater disadvantages are that the method needs fresh semen that this semen must be of good quality and that semen from different individuals may give different results. We have tried to eliminate some of these disadvantages by using the same donor throughout this investigation and by checking his semen continuously

In each case there is an approximate correlation between the agglutinin titre and the immobilization time In case VI the serum had both the highest agglutinin titre and the strongest immobilizing effect. In this case the sperm migration was also much reduced as judged by the postcoital test and the *in vitro* cervical mucus invasion test. Cases IX and XI which had the lowest agglutinin titres, showed the lowest immobilizing effect as well. In these two cases the spermatozoa had greater ability to invade cervical mucus than in the other cases, as was demonstrated both *in vivo* and *in vitro* This indicates *different degrees of sperm immunization* with corresponding influence on the mucus-invading ability of the spermatozoa

In only one case that in which the patient had been operated on for inguinal hernia and also had had an epidydimitis or orchitis can one of the *causes* of autoimmunization against sperm which were mentioned in the introduction be possible In the other 10 cases history and clinical findings did not give any suggestion of the origin of the antibody formation The possibility of spontaneous autoimmunization against sperm is, however in accordance with present theories about the mechanism of autoimmunization (Mackay and Burnet, 1963) As the spermatogenic cells are

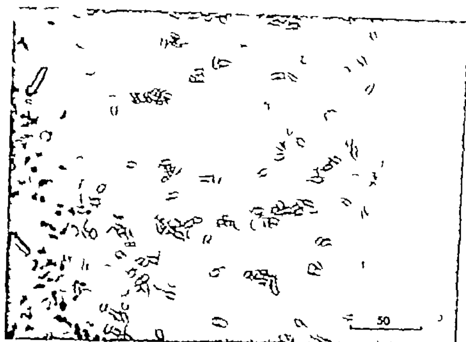


Fig. 7 In vitro cervical mucus invasion test in a case with immunoagglutination. To the left the border of the semen. In the middle of the field spermatozoa have invaded the mucus and halted. To the right mucus free from spermatozoa.

which was observed in the postcoital tests Normal spermatozoa immediately begin to invade ovulatory cervical mucus and may be scattered over the entire mucus mass within a few minutes. In cases of immunoagglutination those spermatozoa which were not agglutinated invaded the cervical mucus but only to a small extent. Then they continued to move *in loco* but gradually became less active Within a few hours all spermatozoa which had invaded the mucus were quite immotile (Fig 7) Cases IX and XI were again exceptions a larger number of spermatozoa invaded the mucus to a greater extent and remained motile for a longer time The tests in these cases were not normal however since most of the spermatozoa stopped moving forward on their way through the mucus so that only a few of them reached the centre of the mucus mass.

ried to another man pregnancy occurred. Common to all the cases was a reduced ability of the spermatozoa to invade ovulatory cervical mucus demonstrated both *in vivo* and *in vitro* Moreover the majority of the spermatozoa in the ejaculates agglutinated, and the agglutinated spermatozoa were not observed to invade the cervical mucus. These facts strongly suggest that immunoagglutination of sperm as judged by the criteria described above is a *cause of sterility*

There are several *other kinds of sperm agglutination*. Slight sperm agglutination is a common phenomenon Some spermatozoa often gather around leucocytes, epithelial cells, or detritus of the semen usually with the head against the foreign cell Immotile spermatozoa can be seen drifting about in large or small congregates. In cases of polyspermia the high sperm density may give a false impression of agglutination Broth cultures of some strains of E. coli are able to agglutinate spermatozoa (Rosenthal, 1931) and some other bacteria have this ability too (Buxton and Wong 1952) Experimentally sperm agglutination can be brought about by various physicochemical agents (Mann 1964) In those cases where antibodies against sperm were demonstrated in the patient's serum the agglutination was more massive and conspicuous than in other cases. Since the heavy agglutination was the only thing that distinguished the ejaculates from normal ones, it is important to notice sperm agglutination during sterility investigations and to test all cases with conspicuous agglutination serologically for antibodies against sperm.

SUMMARY

A report is given of eleven cases of immunoagglutination of sperm. Nine of the cases were found during the investigation of 263 infertile marriages In all the eleven cases the male partners were healthy and had intact genital organs Ten of them showed normal sperm density only one of them a slight oligospermia The antibodies against sperm were revealed because of heavy spontaneous agglutination of sperm which was apparent both macro- and microscopically The agglutinin titres and the sperm-

dormant until a time which is comparatively late in the development of the individual their cell contents might act as "inaccessible antigens to which no tolerance is developed during foetal and early extrauterine life

Semen is chemically and physiologically a very complex material It contains on one hand the spermatozoa on the other the seminal plasma *i e* the secretions of the male accessory glands. The *antigenicity of sperm* is complex too By immunoelectrophoresis of seminal plasma Searcy Craig and Bergqvist (1964) obtained 7 lines of precipitation With the gel diffusion technique of Ouchterlony Rao *et al* (1959 1961) found that human semen contained at least 16 antigens and the spermatozoa 7, of which 4 were the same as those found in seminal plasma With the same technique Bandhauer (1963) demonstrated 8–9 precipitating antigen fractions of seminal plasma and at least 4 of spermatozoa all identical with fractions of the seminal plasma He therefore agreed with Weil *et al* (1956 1960 1961) who demonstrated that spermatozoa acquire antigenic material from the seminal plasma and that these antigens dominate their immunological behaviour However the investigations of Rümke and Hellinga (1959) and Phadke and Padukone (1964) suggest that it must be the testicular spermatozoa which act as antigens in the process of autoimmunization against sperm. A study of which constituent of semen the antibodies are directed against in cases of immunoagglutination would therefore be of interest for the further investigation of the mechanism of autoimmunization against sperm.

The 9 reported cases of immunization against sperm among 263 men in infertile marriages were revealed by the marked spontaneous agglutination in the ejaculates To judge from earlier investigations in this field (Rümke and Hellinga 1959) some cases of sperm antibody formation might be found among the cases of oligo- and azoospermia It is possible therefore that the actual *frequency* of sperm immunization is higher than the 3.4 per cent found in the present investigation

All the 11 marriages were primarily infertile No cause of absolute sterility was found during the investigation of the wives. In both cases where a female partner married or had been mar

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A CASE OF TESTICULAR FEMINIZATION WITH CHROMOSOME MOSAICISM

BY

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Patients with the testicular feminization syndrome are phenotypically females with female external genitalia, a short vagina that ends blindly, lack of uterus and scanty sexual hair. The vagina may be completely absent. The gonads are immature testes; they may be intra-abdominal, situated along the course of the inguinal canal or in the labia majora. The cells lining the tubules are spermatogonia and Sertoli cells (Gordon *et al.* 1964) but spermatogenesis is rare. The Leydig cells may be absent in some areas of the gonads, but in others they may be grouped into adenomatous formations. Hormonal studies usually show normal female or male or sometimes elevated urinary 17-ketosteroids and normal male or female levels of urinary oestrogens. After castration the oestrogen and ketosteroid values decrease and the gonadotrophins increase. This indicates that the gonads are an important hormone producer in these cases. Excellent reviews of the syndrome are given by e.g. Jones and Scott (1958), Netter *et al.* (1958), Hauser (1961), Molnoff and Armstrong (1962), Decourt and Guinet (1962) and Henrion (1963).

The syndrome is hereditary; either an X-linked recessive gene or a sex-limited autosomal dominant gene is involved (*cf.* Philip and Sele 1965). The sex chromosome complement is usually XY (e.g. Hauser 1961, Morris and Mahesh, 1963

immobilizing effect of the mens sera are reported. Common to all the cases was a reduced ability of the spermatozoa to invade ovulatory cervical mucus both on slides and in postcoital tests. Different degrees of sperm immunization with corresponding reduction of the mucus invading ability of the spermatozoa were found. Several facts strongly suggest that the immunoagglutination of sperm is the main cause of sterility in these cases. It is concluded that the finding of heavy agglutination of sperm during investigation of sterile marriages should lead to serological testing of the male partner for antibodies against sperm

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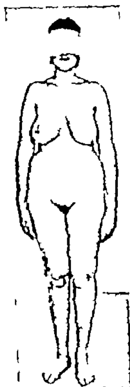


Fig. 1 Photograph of the patient.

found. Thus, in these cells the sex chromosome complement was XYY (Fig.). In the other two cells, the extra chromosome belonged to the group 6–X. According to the buccal smear findings (an average of 8 per cent nuclei contained sex chromatin) this extra chromosome was assumed to be an X-chromosome, the sex chromosome complement being XXY. Thus, most of the cells were of the karyotype 44+XYY, deviating karyotype being found only in a small number of cells, namely four cells with 44+XY and two cells with 44+XXY. These results indicate triple mosaicism XY/XYY/XXY.

Pathological examination of the gonads. The two gonads had the macroscopical appearance of hypoplastic testicles. At the lateral pole there was pronounced cystic formations corresponding to caput epididymidis. Histologically the testicles consisted of undifferentiated tubules. The cells in the epithelium of the tubules had the appearance of Sertoli cells and no germ cells could be seen. The Leydig cells showed hyperplasia. In many regions

Moore *et al* 1964 Court Brown *et al.* 1964) Exceptionally another chromosome complement has been found. Two cases are mentioned by Morris and Mahesh (1963) One patient had an XO/XY/XX mosaic the other was sex chromatin positive In a case of monozygotic twins both the individuals had testicular feminization and an XXY complement (*cf* Miller 1964)

In this paper we describe a patient with testicular feminization and an XY/XYY/XXY sex chromosome mosaic.

Case report The patient, V O was born in 1941 In 1964 she was examined in order to be fitted with a vaginal diaphragm. She seemed to be of low intelligence and admitted that she had experienced difficulty in passing through elementary school. She later attended a vocational school. She has one brother and one sister The sister is an inmate of an institution for the psychically retarded she has a normal (XX) chromosome complement.

The patient is of the opinion that sexually she developed late. She said she had menstruated on two occasions with an interval of approximately three months. However a detailed history revealed that these episodes of bleeding occurred after coitus. She believed herself to be quite healthy

Examination revealed a sturdily-built girl, height 180 cm, with a well-developed pelvic region (see Fig. 1) Her breast development was normal. The general investigation was negative The pubic and axillary hair was scanty The external genitalia were normal there was no clitorial enlargement. The vaginal outlet was marital. The vagina ended blindly it was 8 cm deep. At laparoscopy no uterus or adnexa could be seen. The right gonad was situated at the usual place for an ovary the left gonad was found deeper in the rectouterine pouch.

Intravenous urography showed normal kidneys and normal passage through the ureters. A psychological test showed some debility

At laparotomy the gonads were found to occupy the positions seen at laparoscopy No uterus or adnexa could be seen. Both gonads were removed.

For urinary steroid excretion (24 hours) before and after operation, see Table I.

Chromosome analysis. The technique for blood culture and chromosome preparations was that of Källén and Levan (1962) Buccal smears were fixed in 96 % alcohol and stained in gallocyanin-chromalum. A total of 500 nuclei were studied.

Chromosome counts of cells from two blood cultures showed 47 chromosomes in the majority of the cells and a normal number in the rest. The chromosome counts and the analysis are presented in Table II. All the cells with 46 chromosomes had a normal male karyotype. The Y-chromosome was easily distinguished from the small acrocentric autosomes. In all but two of the cells with 47 chromosomes, two almost identical Y-chromosomes were

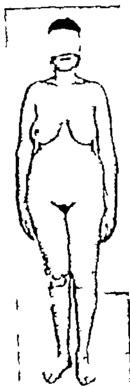


Fig. Photograph of the patient.

found. Thus, in these cells the sex chromosome complement was XYY (Fig.). In the other two cells the extra chromosome belonged to the group 6–12, X. According to the buccal smear findings (an average of 8 per cent nuclei contained sex chromatin) this extra chromosome was assumed to be an X-chromosome the sex chromosome complement being XXY. Thus, most of the cells were of the karyotype 44+XYY deviating karyotype being found only in small number of cells namely four cells with 44+XY and two cells with 44+XXY. These results indicate triple mosaicism XY/XYY/XXY.

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Table I. *Urinary Steroid Excretion (24 hours)*

	Before Operation	After Operation
Pregnandiol	1 7 mg	0.7 mg
Œstrogen	>20 <110 MU	<20 MU
Total gonadotrophin	about 13 MUU	96 MUU
17-ketosteroids	12.6 mg	3.8 mg
Corticosteroids (17-OHCS)	16.4 mg	8.4 mg

Operation Removal of both gonads.

Table II *Chromosome Counts and Sex Chromosomes*

	46 (XY)	47 (XYY)	47 (XXY)
Blood I	3	27	2
Blood II	1	23	0

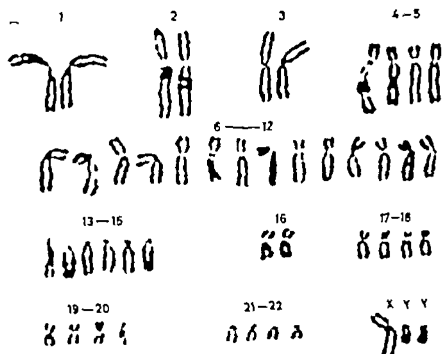


Fig. 2. A karyotype from a blood cell. XYY complement

there were large islets of such cells (Fig. 3) The Leydig cells contained a rich amount of a brown pigment. In the areas with strongly pronounced hyper plasia, the circular-arranged connective tissue around the tubules were in



Fig. 5 Microphotograph showing the marked hyperplasia of the Leydig cells. The circular connective tissue around the tubules is thickened. Magnification, 200 ×

many places thickened and sometimes had a hyaline appearance. Centrally in one of the testicles and peripherally in the other there were delimited regions where the intertubular tissue lacked Leydig cells. Here the tubules were especially crowded and the formations had the appearance of tubular adenomas

Discussion

The patient described presents, from a clinical point of view a typical example of testicular feminization. One exception, however is her low intelligence According to Hauser (1961) most of these patients have an average to high intelligence. More than half of the reported patients have had hernias, with the gonads in the labia majora or inguinal region (Morris and Mahesh, 1963) Our patient had the right gonad in a normal ovarian position, while the left was situated deeper in the rectouterine pouch.

In the present example of testicular feminization, a chromosomal mosaicism exists, and the dominating cell line in peripheral blood has a chromosomal constitution of XYY The XYY consti

tution has not previously been described in such instances. Vignetti *et al* (1964) however describe a 35 year old girl with some features of testicular feminization she had testes in the labia majora and lacked internal female genitalia. Clitoral enlargement was present (2 cm) and in the posterior wall of the urethra a blind pouch was found. Some of the reported patients with an XYY complement have had hypogonadism with or without mental retardation, but there is also an example of a fertile male of average intelligence (*cf* Miller 1964) De Grouchy *et al* (1963) found a probable XYY complement in a patient with Turner s syndrome but the authors do not exclude the possibility of a trisomy-22 Mosaicism of the type XO/XYY has been seen in Turner s syndrome (Jacobs *et al* 1961) and in a patient with pure gonadal dysgenesis (Cooper *et al.* 1962) The latter condition has also been associated with an XO/XY/XYY mosaic (Jones *et al* 1963)

Other types of non-genital pathological conditions have been seen in patients with an XYY complement (*cf* Hustinx and van Olphen, 1963) one case of Marfan s syndrome and one case of Sturge-Weber s syndrome

The XXY sex chromosome set is characteristic for patients with Klinefelter s syndrome but has earlier also been described in a pair of monozygotic twins where both the individuals had this chromosome complement and testicular feminization (*cf* Miller 1964)

In the majority of patients with testicular feminization a normal male chromosome pattern has been found (Jacobs *et al* 1959 Chu *et al* 1960 Puck *et al* 1960 Court Brown *et al* 1964 and others) Therefore the signs of testicular feminization in the present patient are apparently unrelated to the chromosome aberration. This however may be related to the non-typical feature of this patient from the point of view of testicular feminization her low intelligence It is well known that mental subnormality is a common feature of patients with chromosomal aberrations

The explanation of the testicular feminization syndrome is to be sought in an endocrinologic disturbance According to the present-day opinion based on studies by Jost (1958) and others,

the foetal testicle regulates sex development with the aid of two different hormone systems one of classical steroid androgens the other of unknown nature, causes regression of the Müllerian ducts. In patients with testicular feminization, the Müllerian ducts do regress—of the internal genital apparatus only the vagina is left, and this may vary in length considerably On the other hand, no masculinizing effect with development of a prostate seminal vesicles, etc. has been obtained. This would indicate a defect in the production of androgenic steroids in the testicles during foetal development, or possibly an inactivation of the hormone produced, or a lack af activity due to non-responsiveness of the target tissues. In the adult organism, the testes apparently produce androgens as is evidenced by the excretion of 17-ketosteroids, which decrease after castration. Different studies related to the androgen production have shown that the gonads in this syndrome behaves in a fashion similar to the testes in a normal male (Griffiths *et al.* 1963 Barjon *et al.*, 1964) According to Kase and Morris (1965) the androgens are produced in amounts equivalent to the foetal testis.

Some facts argue in favour of the hypothesis of non-action of the testicular androgen. Thus even large doses of androgens are ineffective in producing hair growth and virilization in the adult patient with this syndrome (Morris and Mahesh, 1963)

Naturally it is not possible to base any conclusions regarding the activity of the foetal testes and other tissues upon their behaviour in an adult stage The reasons given above however argue in favour of a non-action of the testicular androgen, this anomaly being inherited but not being due to any gross chromosomal changes.

The testes in these patients also produce oestrogen (*cf* Barjon *et al* 1964) which results in mammary gland development etc. However here also there is often an incomplete reaction to the hormone with poor development of the areolæ and nipples and poor glandular development in spite of a well-developed mammary stroma. Similarly the vulva, and especially the labia minora, are often under-developed (Morris 1953) There are reasons to believe that the oestrogenic hormones are produced in the Leydig cells (Hauser 1961 Barjon *et al.*, 1964)

Even though in the majority of patients with testicular feminization no germ cells can be seen in the testicular tubules on light microscopy of the gonads as was the case in the gonads studied by us electron microscopical observations have revealed the presence of both spermatogonia and Sertoli cells (Gordon *et al.* 1964)

Adenomas are often found in the gonads from these patients. Morris (1953) reports tubular adenomas in approximately 28 per cent of the cases according to Hauser (1961) the figure is about 24 per cent. The danger of malignant tumours in the testicles is probably not above 5–8 per cent (Jones and Scott, 1958 Hauser 1961) even though higher figures have been reported (*cf* Morris and Mahesh 1963)

SUMMARY

The authors describe a case of testicular feminization syndrome with a sex chromosome mosaicism XY/XYY/XXY Most of the blood cells had XYY It is pointed out that testicular feminization is an endocrinological disturbance and that the sex chromosome mosaic in the present instance is apparently unrelated to the syndrome The deviating sex chromosome complement might be related however to the low intelligence of the patient, which is not a typical feature of testicular feminization.

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NECROSIS OF UTERINE MYOMATA

BY

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Uterine myomata are the most frequent gynæcological tumours, occurring in about 15 per cent of all women Myomas develop only during the fertile period and their growth is related to ovarian activity Myomata are most common in the age range 40–50 years more detailed statements on the so-called myoma age have varied greatly On the basis of direction of growth, myomas are classified into three groups the subserous, the intramural and the submucous These tumours otherwise benign often show various degenerative changes and of these necrosis is one of the most important clinically Its incidence varies in different series from 6 to 17 per cent of all myomas treated surgically (Seitz Amreich 1955) for instance Scipiades (1941) reported an incidence of 6 9 per cent and Kuznetsov (1962) 13 5 per cent.

The most common cause of necrosis of a uterine myoma is cessation or impairment of its circulation A myoma may very easily undergo necrosis after delivery or miscarriage when the blood vessels having been dilated undergo rapid constriction (Benthin, 1939 Scipiades 1941) The same applies to the menopause and postmenopausal period when involution of the uterus and of the myoma are disproportional (Björkenheim, 1933) Further the blood supply to a subserous myoma is rapidly occluded by torsion of the pedicle The vascularization of the

myoma as such makes it prone to circulatory disturbances It has only a single artery which may frequently be inadequate for supplying the tumour as a whole (Faulkner 1944) X-irradiation is another possible cause of necrosis, and also though very rarely infection.

To the naked eye a necrotic myoma usually appears of lighter colour than an ordinary myoma and it is of softer consistency An exception is the condition referred to as red degeneration in which most observers assume the necrosis to be due to a hemorrhagic infarct arising as a result of thrombosis the red colour in these cases has been attributed to hæmolysed blood (Faulkner 1947) On histological examination the initial changes in a necrotic myoma are noted in the cell nuclei their outline becomes indistinct and disappears. The cellular membrane disappears and the nucleus stains more faintly Finally only granular or homologous material is seen. Necrosis of a uterine myoma is almost invariably aseptic in the early stages though infection may be superimposed later The symptoms naturally depend upon the extent of the necrosis and thus may be numerous and of great variety Microscopic study of myomas removed surgically has often revealed local necrosis in the absence of significant symptoms. On the other hand rapidly developing extensive necrosis can cause very significant clinical symptoms and require prompt treatment. In the following discussion necrosis of uterine myomata will be considered chiefly from the point of view of the findings at examination.

Material

A total of 769 patients with myomata were treated at the Gynæcological Department of the Central Hospital of Southern Saimaa between March 1955 and October 1964 of these 508 (66 1 per cent) were operated upon. Of these latter 445 (87.6 per cent) were subjected to total or subtotal hysterectomy and 63 (12.4 per cent) were treated by conservative operation (myomectomy) Necrosis was found in 25 (4 9 per cent) of all the myomas surgically removed 12 were subserous myomas (including 4 pedicled) 8 were intramural, and 5 submucous. They varied

from the size of an infant's head (a subserous myoma) to the size and shape of a littlefinger tip (a myoma originating from the cervix) Necrosis was easily recognizable even macroscopically in 12 cases. All diagnoses were verified histologically The average age for patients with necrosis of a myoma was 43 years The oldest was 63 years the youngest 30 Fifteen (60 per cent) had given birth to one or more children

Symptoms Abnormal bleeding pain and subfebrile rise in temperature were the most common symptoms in several cases all three occurred Abnormal bleeding occurred in 14 patients (56 per cent) mostly in the form of metrorrhagia Uterine hæmorrhage is a sign of myomata and does not in itself indicate necrotic change *Twelve patients (48 per cent) complained of* having had pain The degree of pain varied considerably from case to case but was always cramp-like or resembled labour pains. Usually the pain occurred periodically and the patients of their own accord referred to it in terms of labour pain This pain accompanied both the subserous intramural and submucous types. However pain *was definitely related to the extent of* necrosis in 12 cases (48 per cent) definite necrosis was diagnosed *macroscopically and in 8 of these (67 per cent) pain was a* symptom

Three *patients (12 per cent)* had a history of fever (38–39 C) and these patients also had lower abdominal pain resembling labour *pains* In all of these 3 cases a large intramural necrotic myoma was found While definite fever was unusual subfebrile rise in temperature was a very common finding being present in a total of 16 patients (64 per cent) Other isolated symptoms were compression due to the myoma frequency of urination and in one case nausea and vomiting

Laboratory investigations The erythrocyte sedimentation rate was definitely increased in almost all of the cases There were only 2 patients with a SR below 10 mm/hr and in each of them necrosis was only diagnosed on microscopic examination The maximum SR was 106 mm/hr and the mean 44 mm/hr

An increase in the leucocyte count is also considered typical of necrosis of a myoma The highest count in this study was 13 900 However the mean 7073 was within normal limits. *Only 6*

patients showed leucocyte values exceeding 9000 In five of these necrosis was distinctly recognizable even macroscopically

Haemoglobin averaged 11.4 g per 100 ml the lowest value was 7.4 g per 100 ml. Abnormal bleeding occurred in slightly over half of the cases, in most of these only for a short period before admission and thus no serious haemorrhagic anaemia had usually developed. Often it was a symptom other than bleeding that made the patients first seek medical attention

Two unusual cases will be reported below

Case Record No. 3649/57 The patient was nullipara, aged 63 The menopause had occurred at the age of 49, and had been followed by bloody vaginal discharge. For about one month she had had frequent attacks of lower abdominal pain The periodic pain was associated with nausea and vomiting, fatigue and loss of weight. Gynaecological examination showed that there was in the lower abdomen a firm, fairly mobile mass extending to two finger-breadths below the umbilicus Axillary temperature was 37.6° C., SR 25 mm/hr leucocyte count 00 blood pressure 40/90 mm Hg. Laparotomy revealed smooth tumour the size of an infant' head and adherent to the anterior wall of the abdominal cavity on the right side. The tumour having a very short pedicle was found to start from the right cornu of the myomatous uterus, which had rotated once round its axis. Subtotal hysterectomy was performed The tumour contained disintegrating material and slight amount of turbid fluid. Histological diagnosis was Necrotic fibromyoma.

In this case the necrosis of the myoma had been caused by unusual rotation of the entire uterus, which occluded the blood supply to the myoma. The symptoms were probably attributable to the torsion and the consequent peritoneal irritation, as well as to necrosis of the myoma

Case 2. Record No 754/ 957 The patient was 36 years old Menstrual periods had been normal, cycle 28 days, duration 5–6 days. She had had no earlier gynaecological diseases She was delivered on Oct. 3 956 On post partum follow-up examination mass bout the size of first and attached to the right cornu uteri, was found Re-examination one month later showed that the mass had enlarged, and the patient was admitted for operation on January 23, 957 At the time the axillary temperature was 37.2 C, SR 22 mm/hr leucocyte ount 0,300 At laparotomy the floor of the pelvis was found to be covered by omental and intestinal adhesions The small intestine and the caecum were firmly adherent to the fundus uteri. The uterus was of usual size and its right cornu was the site of origin of capsule-covered tumour the size of large apple and containing disintegrating material, the tumour was enucleated Study of the rough surfaces of the freed intestine showed that the appendix, the thickness of little finger was adherent longitudinally to the ileum. The appendix was removed. The

mucous membrane of the opened appendix was thickened throughout and reddened as a result of acute inflammation. The removed tumour was a necrotic myoma in process of disintegration. Histological diagnosis was Necrotic infected fibromyoma. The material for microscopic study included a piece of omentum, which was also found to be infected.

The myoma had probably first undergone necrosis during the puerperium, and then extension of infection from acute appendicitis had occurred in addition. Infection is rare especially in subserous myomata.

Treatment and results. Total or subtotal hysterectomy had been performed in 22 cases and in 2 cases conservative operation only (myomectomy) There was one polypoid necrotic myoma, the size of a little finger originating from the portio uteri the myoma only was removed There were no deaths. No wound infections or other postoperative complications occurred. In cases where a necrotic myoma caused fever or a subfebrile rise in temperature the temperature returned to normal a few days after operation

Discussion

Necrosis was found in 4 9 per cent of the myomas treated surgically which may be considered a small proportion compared with the figures usually reported. The most common symptoms of these patients were lower abdominal pain abnormal bleeding, and subfebrile temperature rise Hæmorrhages as such are a sign of myomata but pain is not generally present in the case of an ordinary myoma Pain was a symptom in 48 per cent of the patients and it usually resembled labour pain and was of colicky nature There was a definite correlation between the extent of necrosis and the pain pain occurred in 67 per cent of the cases when necrosis was evident macroscopically The pain is probably due in the main to peritoneal irritation but tension in the myoma capsule and reflex contractions of uterine muscle probably play a part (Voelcker 1925) Subfebrile rise in temperature was present frequently in 64 per cent of the patients only 12 per cent had fever exceeding 38 C Thus altogether increased temperature was recorded for 76 per cent and it was evidently due to resorption of necrotic tissue Almost all patients showed a distinct increase in SR the mean being 44 mm/hr A myoma of the ordinary kind does not cause a rise in SR So a definitely in-

creased SR in association with myomata must be considered an important sign of necrosis in the absence of other possible causes of increased SR. The haemoglobin values were on average normal Distinct leucocytosis was demonstrated in the cases with the most extensive necroses.

The two cases reported in greater detail include one of necrosis of a myoma due to rotation of the entire uterus the other is a rare example of infection of a subserous necrotic myoma.

SUMMARY

In a series of 508 myomas treated surgically 25 (4.9 per cent) were found to be necrotic. The most frequent symptoms were a subfebrile temperature rise and lower abdominal pain. The sedimentation rate was definitely increased in almost all cases. Marked leucocytosis was noted in connection with the most extensive necroses.

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SEASONAL CHANGES IN OBSTETRICAL PHENOMENA[1]

BY

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The seasonal variations in disease reflect a) the internal biological rhythm which originally developed to guarantee the preservation of the species (the rhythm is usually related to sunlight or temperature) b) secondary stress phenomena influenced by season (e.g infections, different working conditions) c) seasonal phenomena caused by customs (festivals, variation in diet etc., based on tradition)

The authors have earlier investigated the seasonal variation in the number of births and in cystic glandular hyperplasia, and found a correlation with luminosity but not with temperature (Timonen *et al* 1964)

The aim of this study is to examine seasonal phenomena related to pregnancy and childbirth, in the hope that such an examination will help in understanding the reasons for obstetrical complications and perhaps will aid also in planning prophylactic measures.

Material and Methods

The material consists of two series 27 522 deliveries in Department II of Obstetrics and Gynaecology Helsinki University Central Hospital, from the years 1951 1960 (Series 1) and all de-

[1] This investigation was aided by grant from the Sigrid Juselius Foundation

T bl I Seasonal hanges in th conception t South and North Fi land sepa at ly The i crease i con eptions duri g the light period is highly sign fic t I Northern Finland p gnancies with mal fa t increased mo e tha pregnanci s with female inf t (χ^2 = 5 56*) Series 2

Months of Conception	South Finland						North Finland					
	No of Births Months Later			Per Cent			No of Births Months Later			Per Cent		
	Male infants	Female infants	Total	Male infants	Female infants	Total	Male infants	Female infants	Total	Male infants	Female infants	Total
VI—XI (light)	3824	3589	274 3	5 9	53 3	53	2533	2358	4891	53 1	5 3	52 7
XII—V (dark)	84	1866	4 5	47	46 7	46 9	2 36	2146	438	46 9	47 7	47 3

liveries in Finnish maternity hospitals during the period July 1 1957–June 30 1958 totalling 57 089 (Series 2) Multiple births and deliveries in diabetic women were omitted from all tables, and pregnancies resulting in stillbirths or malformed infants were omitted from some tables. All other pregnancies of ≥134 days duration and resulting in the birth of a child of ≥600 g were in cluded.

The constitution of the child was studied by dividing the new-born into the following groups

Stout height (cm)—34≤weight (g) 0 36—4
Slender height (cm)—34≥weight (g) 0 36—4

These relations were chosen in such a way that the ratio *stout/slender* among all the newborn studied was about 1 1

Statistical significance was determined by the chi-square test. When no figures are given ≥99 9 per cent signficance is indicated by *three asterisks* 99 9 *per cent signficance* by *two and* 99–95 per cent significance by one asterisk

Conception Rate

In an earlier investigation we compared luminosity temperature and conception rate (Timonen *et al* 1964) Luminosity was significantly correlated with conception rate but temperature had *no effect* Seasonal variation of conception rate was studied recently by Takahashi (1964) He found a midsummer peak *in subfrigid climates* but explained it by a favourable average temperature and did not correlate his results with luminosity

In the present study (Series 2 whole country) we have checked our earlier results by comparing the effect of the light and dark periods of the year (Table I) The conception time is regarded as being 9 months before delivery The figures have been adjusted to compensate for the fact that the duration of months VI–IX exceeds that of months XII–V by one day Since we have noted *earlier* that the effect of light on the LH-secretion of the hypophysis becomes evident after a latent period of about one month we have combined the months VI–IX instead of V–X, when the amount of sunlight is highest versus XII–V instead of XI–IV The difference between these groups was once more highly signifi-

				Girl						
34—38	88	37	90	3	8	79	2.05	35	1 37	57
39—45	3	83	87	3	85	37	6	89	81	0.71
46—5	67	70	69		48	37	20	75	3	1 15
53—259	38	57	40	5	3 3	2 3	75	2 76	5	2 8
60—266	6 43	5 64	6 7	5 73	6 48	6 99	6 98	5 73	6.34	6 44
267—273	7 49	5 46	7 35	5	7 78	5 55	7 3	15 6	7 86	15 76
74—80	26 4	4 59	7 88	8 6	7 6	24 84	26 27	25 63	26.88	26 6
8—87	5 09	26 2	4 77	5	23 98	3 58	24 00	26 14	25 4	24 50
288—294	1 44	4 59	9	3 7	53	4 65	1 48	3 78	56	3.5
295—30	4 89	5 7	3 98	5 78	4 15	5 46	4 3	4 75	4 56	6 5
302—	46	87	53	66	8	2	1 59	1 96	1 59	1 78
T tal um be of case	395	24	833	1868	1760	904	2767	758	3200	3383

Table II *Seasonal variation in the duration of pregnancy The numbers give the percentages of pregnancies in different duration groups Estimations of significance of the differences in the groups showing most variations from the mean are as follows*

Mothers aged < 25 male infants months V—X versus XI—IV χ^2 = 44 8
Mothers aged > 25 male infants months V—X versus XI—IV χ^2 = 44 8
Para 3 male infants months V—X versus XI—IV χ^2 = 27 3**
Para 2 female infants months V—X versus XI—IV χ^2 = 23 3
Mothers < 25 female infants months V—X versus XI—IV χ^2 = 23 1

Series 1

Duration of Pregnancy in Days	I—p		II—p		III—p		< 25		> 25	
	XI—IV	V—X	XI—IV	V—X	XI—IV	V—X	XI—IV	V—X	XI—IV	V—X
				Boys						
134—238	2 06	2 00	1 12	1 12	1 73	1 63	1 80	1 95	1 47	1 45
239—245	1 32	0 90	1 12	0 41	1 39	0 63	1 58	0 72	1 02	0 64
246—252	2 06	1 68	1 83	1 48	1 67	1 21	2 04	1 67	1 73	1 37
253—259	3 45	3 08	2 54	2 60	3 35	3 22	3 41	3 40	2 90	2 70
260—266	7 93	7 13	7 74	7 34	8 76	7 28	7 88	7 50	8 25	7 23
267—273	17 76	15 95	20 10	16 42	16 34	14 83	18 76	13 80	17 54	15 43
274—280	25 81	25 17	26 31	27 03	26 94	23 85	26 58	26 48	26 09	24 99
281—287	22 03	23 58	22 44	23 20	22 31	26 23	20 84	24 19	23 46	24 96
288—294	11 59	13 74	11 30	13 62	10 60	13 35	11 30	13 65	11 15	13 89
295—301	4 56	4 96	3 82	4 74	4 57	5 12	4 22	4 53	4 42	5 20
302—	44	1 81	1 68	1 94	2 34	2 64	1 49	2 10	1 97	2 15
Total numbe of cases	2433	2439	1965	1961	1793	1895	2841	2761	3346	3442

T bl III *Seasonal changes in the duration f pregnancy in South East and North Finland. There is a signific nt prolongation f pregnancy during the light period in South Finland* (χ^2 = 28 8**) *Series 2*

Duration of Pregnancy in Days	South Finland		East Finland		North Finland	
	XI—IV	V—X	XI—IV	V—X	XI—IV	V—X
34—238	.68	74	1 77	1.8	1 79	1 68
39—245	0 96	0 92	0.90	0.87	0 92	1 13
246—252	68	8	2.07	66	93	80
53—259	3 7	94	2.55	2 58	3 00	3 05
260—266	6.30	5.8	6 09	6 23	6 43	6 22
267— 73	14 12	13.27	13 96	15 07	4 7	13 91
274— 80	24 96	4 24	5.24	24 72	5.85	5 18
8 —287	25 20	24 95	24 8	25 33	22 93	23 68
88—294	3 59	4 35	3.67	3 62	2 99	13 37
295—30	5	6.00	5 29	4 76	5.11	5.2
3 2—	3 30	3 77	3 65	3 34	4 79	4 76
T tal number of cases	7536	8893	3760	3802	4465	5047

The Duration of Labour

There are no marked seasonal variations in the duration of labour but there is a slight tendency to more rapid labours in July August. The second stage of labour in primigravidae with male infants is significantly shorter in summer (Table V) and in multigravidae with infants of either sex the difference is highly significant.

If we consider the data in Tables II and VI in relation to the possible influence of the function of the posterior lobe of the hypophysis we are faced with two opposite trends in summertime the duration of pregnancy lengthens and prolonged pregnancy becomes more frequent, but labour especially the second stage becomes shorter Although it is very tempting to assume a connection between the lengthening of the duration of pregnancy during summertime and the negative relation of the oxytocin release mechanism to light or temperature, not all seasonal variations of labour can be explained on the basis of this single

cant. Contrary to our expectation the difference between North and South Finland was not significant. It seems however that conceptions of males were significantly (98 per cent) higher in summer than in winter in North Finland. Comparison of our results with those reported by others indicates that the conception rate rises later in the year in Finland than in Central Europe where the peak is reached in June (Otto 1959)

Duration of Pregnancy

Pregnancies terminating in May–October tend to be prolonged (Table II) This trend varies in degree in different age and parity groups and is particularly marked in association with male infants With female infants the phenomenon is significant only in the younger age group This result is, in our opinion noteworthy hitherto no accurate statistical studies have been carried out (Tromp 1963) although there has been lively interest in this problem (Kuestner 1931 Knorre, 1933 Nordmeyer 1937 Wahl 1938 Bernhart 1938)

If we compare the frequencies of postmaturity (from 302 days onwards) in Table II we notice that the figures are always higher in the light period. For example, in young mothers with male infants the frequencies are 2.64 against 1.49 per cent, and in mothers over 25 years with male infants 2 15 against 1 97 per cent. With female infants the differences show the same trend. This tendency is not great enough to be of any practical importance, but it must be regarded as an established biological phenomenon. It becomes more obvious when the frequencies of distributions in different duration groups of pregnancy are compared (Table II) Prematurity shows no significant seasonal changes con firming an observation made previously by Otto (1959)

When the seasonal changes in different regions were studied the only notable variations were in South Finland (Table III) It therefore seems that women are more susceptible to seasonal stress in South than in North Finland. If light has a negative feed back effect on oxytocin secretion, the seasonal variations should be accentuated in North Finland, where during summertime there is practically no night and during winter only night or twilight.

Month of Delivery	Primigravidae									
	Male Infants					Female Infants				
	Percentages of Cases				No. of Cases	Percentages of Cases				No. of Cases
	Duration of Labour in Hours					Duration of Labour in Hours				
	0—	—	24—48	Over 48		0—15	5—24	24—48	Over 48	
I—II	43 6	3	20 8	4 4	727	4 6	30 7	23	7 5	695
III—IV	4 8	29 8	4	3 4	865	42 2	28 7	23 8	5 3	910
V—VI	43 8	29 9	20 9	4 4	8 4	42 7	3 9	2 5	1 9	830
VII—VIII	43	32.3	3	3	758	44	30.6	2 5	3 9	763
IX—X	4 7	3	24	3	726	45 7	29.5	20.7	4	706
XI—XII	47	28 3	5	3	683	4 5	32 3	3 3	2 9	678
Whole year	43 6	30 7		3 5	4573	43 1	30.7	2 6	3 6	4582

Table IV *Seasonal variation in the duration of labour in primigravidæ and multigravidæ Series 1 The increase in duration in the group < 15 hrs in July-August is almost significant (χ^2 = 11 63) in multigravidæ with male infants The trend is the same in all groups except in primigravidæ with male infants*

Month of Delivery	Multigravidæ							
	Male Infants				Female Infant			
	Percentages of Cases			No of Cases	Percentages of Cases			No. of Cases
	Duration of Labour in Hours				Duration of Labour in Hours			
	0— 5	5—24	Over 24		0—15	5—24	Over 4	
I—II	73 2	18 0	8 7	1179	73 7	18 3	8 0	1105
III—IV	71 6	19 4	8 9	1352	72 5	17 7	9 8	1305
V—VI	75 0	16 7	8 2	1312	75 1	17 1	7 8	1261
VII—VIII	77 0	16 3	6 5	1283	76 0	17 7	6 3	1198
IX—X	72 9	19 0	8 0	1120	73 3	19 0	7 7	1140
XI—XII	75 0	17 0	8 3	1075	72 5	19 1	8 4	1023
Whole year	74 2	17 7	8 1	7321	73 9	18 2	7 9	7032

Month of Delivery	Multigravidae							
	Male Infants				Female Infants			
	Percentages of Cases			No. of Cases	Percentages of Cases			No. of Cases
	Duration of the Second Stage in Minutes				Duration of the Second Stage in Minutes			
	0—4	5—35	Over 60		0—4	5—35	Over 60	
I—II	76	9 4	4 5	39	75	8 5	6	076
III—IV	67 7	24 7	7 4	3 7	68	25 8	5 9	1266
V—VI	79 8	7 4	2 7	46	78 7	18 1	3	202
VII—VIII	79	16 3	4 3	204	81 5	15	3 4	1134
IX—X	7 4	1	7 4	79	74 7	9 4	6 0	1096
XI—XII	76.7	8 2	5 0	44	78 6	7 3	4	998
Whole year	75.	9 6	5 2	7039	76.	9.2	4 8	6772

Table V *Seasonal variation in the duration of the second stage of labour The shortening of this stage in July-August is highly significant in all groups of parturients Series 1*

Month of Delivery	Primigravidae									
	Male Infants					Female Infants				
	Percentages of Cases				No. of Cases	Percentages of Cases				No. of Cases
	Duration of the Second Stage in Minutes					Duration of the Second Stage in Minutes				
	0—15	5—35	35—60	Over 60		0—5	5—35	35—60	Over 60	
I—II	24 9	44 5	19·5	11 1	714	23 2	47 5	18 18	11 1	673
III—IV	21 6	43 7	23 7	10 9	852	26 4	45 0	18 3	10 5	894
V—VI	26 0	44 7	19 7	9 5	776	29 1	45 4	17 3	8 2	791
VII—VIII	27 5	44 4	18 3	8 4	746	30 5	43 4	18 1	8 3	727
IX—X	20 9	45 7	20 3	12 8	704	28 8	40 3	18 0	12 9	680
XI—XII	26 9	45 1	14 6	12 9	665	30 3	43 5	17 3	8 9	664
Whole year	24 6	44 8	19 5	10 9	4457	28 0	44 2	17 8	10 0	4429

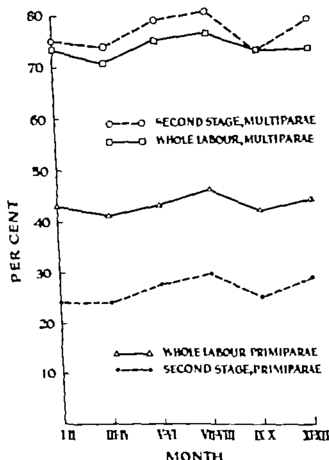


Fig. Seasonal changes in the duration of the whole of labour (percentage of cases < 5 hrs) and of the second stage of labour (percentage of cases < 5 min.) Series. The peak in all groups studied in July–August.

aetiology makes the evaluation of the results difficult. It is even possible that they are complicated by psychological factors arising from light and temperature conditions.

Toxaemia of Late Pregnancy

The seasonal variations in the frequency of late pregnancy toxaemia were examined geographically (Table VII). The figures

Table VI *Seasonal variation of uterine dystocias (primary and secondary inertia) The difference between March April and July-August is highly significant Series 1*

Month of Delivery	No. of Cases	Total No. of Cases	Per Cent
I—II	54	3602	1 50
III—IV	104	4339	2 40
V—VI	62	4015	1 54
VII—VIII	41	3811	1 08
IX—X	51	3559	1 43
XI—XII	63	3371	1 87
Whole year	375	22697	1 65

factor The shortening of the duration of the second stage and perhaps of the whole of labour during summertime should perhaps be attributed to variations in general health. As a result of better general health the co-ordination of labour might be more effective during summertime, when both the dietary and psychological conditions are better than in winter This becomes evident from the fact that in late summer (months VII–VIII) there are fewer disorders in uterine contractility than at other seasons especially spring (months III–IV) (Table VI)

Hyperemesis

The occurrence of hyperemesis as estimated from the number of patients admitted to hospital, was studied from the standpoint of seasonal variation The data covered a 10-year period (Series 1) Figure 2 gives the frequency of the cases. It can be seen that during the summer months the incidence is lower than during the winter, the minimum being in months IV–V Thus hyperemesis differs from late pregnancy toxaemia where the lowest incidence is not reached until the late summer and where no delayed effect attributable to luminosity can be seen. The obscure and complex

N B The close connection f the oxytocin release system to the secretion of antidiuretic hormone the well-known diurnal variation of urine excretion and the increased frequencies of deliveries during the night (Johansson)

*T ble VII Seasonal hanges in h frequency of toxaemia of lat pr gnancy in different parts of F nland The country was divided into he southern, orthern western and eastern part Th apital (Helsinki) and two other big ci es (Turku and Tampere) were considered separately The frequency of toxaemia varies in different parts of the country owing t the different criteria employed. The lowest frequency in the whole country in July-August is highly gnificant (= 24 7***)*

	Months I II			Months III IV			Months V VI		
	Total No of Cases	Toxae-mias	Per Cent	Total No of Cases	Toxae-mias	Per Cent	Total No of Cases	Tox mias	Per Cent
Helsinki	33	93	7	580	98	6	1465	98	6.7
T + Tam	403	60	4 9	9 7	66	7	805	65	8 1
South	6 2	48	3	97	6	3	983	53	2 7
West	893	5	7	48	83	3 7	2069	58	8
East	74	39	3 3	3	4	3	23	54	4 4
North	87	10	3	455	32	2	1478	44	3 0
T t l	768	3	4	9483	352	3.7	903	37	4 1

	Months VII VIII			Months IX X			Months XI-XII		
	Total No of Cases	Toxa mias	Per Cent	Total No of Cas s	Toxa-mias	Per Cent	Total No of Cases	Toxa-mias	Per Cent
H lsinki	2265	108	4 8	965	06	5 4	294	76	5 9
T + T m	397	55	3 9	401	9	23 0	408	73	18 0
South	676	46	2 7	1546	52	3.4	63	48	2 9
West	983	34	1 7	1849	45	2 4	898	54	8
East	210	48	3 9	197	53	4 4	1 65	3	2 7
North	1058	7	9	670	40	4	1416	41	2 9
T t l	9499	308	3.2	8837	388	4 5	7709	324	4

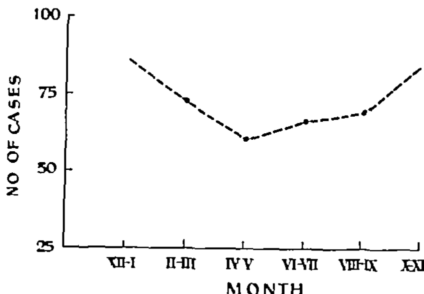


Fig. 2 Seasonal frequency of cases with hyperemesis of early pregnancy treated in Department II of Obstetrics and Gynaecology Helsinki University Central Hospital during 1952–1962 The decrease during months IV–IX is highly significant.

varied greatly from one area to another owing to the different diagnostic criteria employed. The small amount of data from the different parts of the country further increased the difficulty of evaluating the geographical factors. The incidence of late pregnancy toxaemia has been accurately examined previously (Pelkonen 1943) Evidently the differences in climate in different countries lead to different types of seasonal variations (Hosemann 1953 Tromp 1963)

The low incidence of toxaemia in Finland in summertime is explained by the positive correlation between toxaemia and viral infections the occurrence of viral infections is lowest in the late summer (Schroderus, 1929 Pelkonen, 1943)

The Shape of the Newborn

In a previous study we have noticed that the children born in East and North Finland are slenderer than those born in West and South Finland (Timonen *et al* in press) Here we have paid attention to the seasonal variation of the child's shape

T bl VIII Seasonal and g g phical ha g i th slenderness of th newborn Both boys and girls born in th late summer h have been slenderer th n those born in winter. This is especially marked wh th whole country is considered. Not the slenderness of the newborn in East and Northern Finland compared with other parts of th country.

Month of Delivery	Helsinki No. of New-born	Helsinki Per cent Smart	Turku Tampere No. of New-born	Turku Tampere Per cent Smart	South Finland No. of New-born	South Finland Per cent Smart	West Finland No. of New-born	West Finland Per Cent Smart	East Finland No. of New-born	East Finland Per Cent Smart	North Finland No. of New-born	North Finland Per Cent Smart	Whole country No. of New-born	Whole country Per Cent Smart
Male infants														
I—II	667	5	206	55 3	8	5	95	50 6	603	4 8	649	4 0	3887	48 1
III—IV	8 7	55	49	54 4	5	46	43	48 6	72	45 3	736	40 2	4932	48.0
V—VI	77	49 9	4	54	008	48	2	54 7	6 6	44 6	785	43 3	46 2	48 8
VII—VIII	20	49 3	90	47 9	846	5 5	3	48 8	6	4 3	2	39.4	4870	46.
IX—X	983	45	20	55	785	50	939	49 6	653	43	808	42 9	4368	46 7
XI—XII	679	53		54	798	5 5	958	53 4	603	48 9	779	4 5	4 29	50 2
Total	5 20	50 4	7	53 7	5 74	49	6024	50 9	3805	44	4769	4 4	26708	47 9
Female infants														
I—II	665	56 8	97	67 0	800	59 5	943	55 4	57	5 8	638	54 6	38 4	56 4
III—IV	63	60 8	4 6	62 4	947	60 2	5	57 3	59	5 8	7 9	55 5	455	58 0
V—VI	693	5 7	385	60 5	975	5	1 48	58 9	6 5	5	693	52 4	4409	54 4
VII—VIII	063	54 3	207	64 7	83	55 4	973	54	61	48 8	946	50.1	4629	53 4
IX—X	984	54 3	99	58 3	761	55 8	9 0	56 5	544	60.1	87	51 3	4272	55 3
XI—XII	615	57	94	57 7	832	58	940	55 6	562	54 6	637	54 6	3780	56
Total	4783	55 8	608	61 7	5 45	56 6	59 0	56 3	3493	53.0	45 4	52 8	25455	55 5

(Table VIII) A highly significant drop in the frequency of stoutness is found in both girls and boys during July–August. In East and North Finland where the frequency of stout babies is low anyway the seasonal variation was not so marked as in the big cities, especially Helsinki Turku and Tampere. From Table VIII it can further be seen that the seasonal variation is highly significant in both sexes but is especially marked in girls It is impossible within the limits of this study to determine what factors are responsible for the observed seasonal variation in shape. Considering earlier observations that a low frequency of stoutness is connected with a low standard of living and with certain complications of delivery e g toxemia we are inclined to consider the former as an unfavourable factor On the other hand, we have discovered (Timonen et al in press) that in the weight group 3500–3999 g, the babies are slenderer than in the lower or higher weight groups This suggests that between the increase in height and the gain in weight of the child there exists a certain equilibrium which, in normal pregnancy and in optimal circumstances would lead to an acceleration of the child s gain in height One would thus expect that summertime would provide the best conditions for a spurt to a final growth increase. It has been found earlier in Norway that the weight of the newborn varies according to the season children born in summer being heavier than those born during the winter (Kirsten, 1933)

In Vienna (Abels 1922) the weight maximum occurred in June–July and in Odessa in August–October (Gerschenson, 1931) In both series the weight increase started in April In this study we have not considered the variation in weight but in view of the above-mentioned consistent results it may be concluded that the decrease in the frequency of stoutness during late summertime does not imply a deterioration in the nutrition of the foetus but more probably an amelioration which is accompanied by a great increase in height as well as an increase in weight, the increase in height, however being the dominant phenomenon.

Sex Ratio

The sex ratio (boys/girls) was examined in Series 2 and marked variations were found only in South Finland (Table IX)

T bl X Se sonal ha ges asphyxia f h where There is an overall sign fic tr d away f m asphyxia in J ly-A gust in non-toxaemic pr gnancie Th boys significantly mor asphyxiat d than th girls d ri g th wi ter season (χ^2 = 4 9***).

Month of Delivery	Male Infants									Female Infants					
	Non-tox			Tox			Non-tox Tox			Non tox			Tox		
	No of cases	Per cent	Contr	No of Cases	Per cent	Contr	No of Cases	Per Cent	Contr	No of Cases	Per Cent	Contr	No of Cases	Per Cent	Contr
I—II	58	9	987	20	7	297	79	3 5	84	4	3	903	1	3 7	71
III—IV	7	3	336		6	3	9	3 5	2658	70	4	2310	13	4	315
V—VI		3	3		7 3	300	94	3 7	25 3	54	2	223	4	5 6	251
VII—VIII	5	3	35		4 9	43	6	6	2378	43	7	2033	3	6 7	94
IX—X	5	6	937	4	4 8	290	64	9	7	32		19 8	8	3	256
XI—XII	64	3 4	868	6	6 8	237	80	3 8	05	35		775		5	35
Total	366	9	486	5	6	689	47	3 3	4 75	276	3	2 72	70	4 6	15 2

Month of Delivery	Female Infants			Male Female Infants								
	Non Tox Tox			Non-tox			Tox			Non-tox Tox		
	No of Cases	Per Cent	Contr	No of Cases	Per Cent	Contr	No of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr
I—II	5	4	174	00	6	3890	3	5 5	568	13	9	4458
III—IV	83	3	6 5	42	3 1	4646	33	5	637	175	3 3	5283
V—VI	68	2 7	2474	26	2 8	4446	36	6 5	551	16	3	4997
VII—VIII	56	2 5	2 27	93	2 2	4 68	25	5 7	437	18	2 6	4605
IX—X	40	8	2 84	82	1	3865	2	4	546	104	2 4	4411
XI—XII	47	3	2010	99	2 7	3642	8	5 9	472	1 7	3 1	4115
Total	346	5	13694	642	2 6	24658	175	5 5	3 1	8 7	9	27869

Table IX *Monthly variations of sex ratio in South Finland Conceptions in March April and September October give the lowest proportions of male infants* (χ^2 = 31 2)

		Male Infants		Female Infants		Total
Month of Delivery	Month of Conception	No of Cases	Per cent	No. of Cases	Per Cent	No. of Case
I—II	IV—V	2581	50 4	2549	49 6	5130
III—IV	VI—VII	2809	46 8	3193	53 2	6002
V—VI	VIII—IX	3178	51 1	3043	48 9	6221
VII—VIII	X—XI	3220	51 0	3097	49 0	6317
IX—X	XII—I	2879	49 7	2904	50 3	5783
XI—XII	II —III	2569	51 0	2466	49.0	5035
Whole year		17236		17752		34488

Earlier we have reported that the sex ratio was more biassed in favour of males in East and North Finland than in West and South Finland (Timonen *et al* in press) The seasonal variation appeared to include two peaks the lowest proportions of boys were in March–April and in September–October These changes were highly significant. According to the literature a double-peaked sex ratio has also been found elsewhere For example, Petersen (1947) in Chicago stated that the frequency of boys increased if there was stress resulting from either a cold or a hot spell at the time of conception. We found a greater preponderance of girls in pregnancies commencing in June–July and in December–January

The variation of the sex ratio has been related to thyrotropin production (Riddle *et al* 1925) a high thyrotropin excretion resulting in an excess of male offspring Cold stress causes an increase in thyrotropin excretion (Uotila 1939) and this in turn would accelerate the cortisol turnover This leads to an increased excretion of tetrahydrocortisone (Okamoto *et al* 1964) resulting in elevated 17-OHCS levels in the urine Watanabe (1964) states that extremes of temperature increased activity of the adrenal cortex especially in winter

In Finland the coldest winter month is February and there is hardly a "heat stress period. The summer can be considered to be the most favourable season as regards temperature. The pre-

T bl XI Neonatal fa alic Th decre se Mar-Jun i highly s ific (χ^2 = 9 ***) i ne ly all groups studied Seri

Month of Delivery	Non-tox									Non-tox + Tox.		
	Male Infants			Female Infants			Male Female Infants			Male + Female Infants		
	No. of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr
I—II	20	6	98	74	3 9	903	94	5	3890	2 9	4 9	4458
III—IV	48		336	46		3	94		4646	00	9	5283
V—VI	20	9	3		5	2 3	3	7	4446	36	7	4997
VII—VIII	4	9	35	3	5	2033	7	7	4 68	78	7	4605
IX—X	58	3	937	55	9	9 8	3	9	3865	12	8	441
XI—XII	59	3	868	46	6	775	05	9	3643	6	8	41 5
Total	346	8	2486	263		72	609	5	4638	67	4	27869

Month of Delivery	Tox...								
	Male Infants			Female Infants			Male Female Infants		
	No. of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr	No. of Cases	Per Cent	Contr
I—II	4	4 7	297	1	4	27	25	4 4	568
III—IV		3	3 2	5	6	3 5	6	9	637
V—VI	3	1	300		4	5	4	0.7	551
VII—VIII	4	1 6	43	3	1 5	194	7	6	437
IX—X	4	1 4	90	5	2	256	9	1 6	546
XI—XII	8	3 4	37	3	3	235	1	3	47
Total	34	2	689	28	1 8	15 2	62	1 9	3211

ponderance of female infants in pregnancies commencing during the summer is thus understandable and agrees with Petersen's Chicago data. By contrast the cold stress is of long duration and its effect is difficult to analyse The preponderance of female infants in pregnancies commencing in December–January can perhaps be explained by the fact that in February and March there are more abortions than at other times. As is well known, in abortions more males are lost than females Thus the only primary change in the sex ratio is the preponderance of girls in pregnancies commencing during the summer

Asphyxia of the Newborn

As an indicator we have selected only one condition diagnosed among the live-born and surviving children. This is known in our clinic as anoxia without organic lesions This diagnosis implies that at birth the child is so gravely asphyxiated that it has to be kept in a raised oxygen atmosphere for more than 1 h., but that no specific lesions are subsequently found. A more detailed study concerning lesions induced by anoxia and malformations has been made in another connection In non toxæmic pregnancies there was a significant seasonal variation, the peak of which was in March–April and a definite decrease in September–October (Table X) In the literature attention has been drawn to the high perinatal mortality during early spring (Pankamaa, 1955) and the higher frequency of deliveries amongst the mentally deficient and schizophrenics during the spring (Tramer 1929 Knobloch *et al* 1958 Nolting (Sauvage) 1951) The condition that we are considering here does not show a clear increase until the late spring, which suggests that the causes of this type of asphyxia have a somewhat later seasonal rhythm in Finland than in Central Europe, a fact which is also evident in many other obstetrical phenomena that show seasonal variations.

Recovery from the fatigue caused by the long winter season evidently takes several months There was a higly significant increase in asphyxiated male infants during the whole winter period.

The elimination of the sex bias in asphyxia during the summer

T ble XII *Seasonal f eq ency f postpart m infections in series 1 (only non-toxaemic parturients) A highly significant increase of puerperal terine infections can be seen d ring th lat winter period (χ^2 = 36 — 60**). In fection of the mammary gland increased in the first half of th year (χ^2 = 34***) Infection f the urinary t act decreased almost significantly (χ^2 = 12 4*) d ring the summer (May-A gust)*

Male + Female Infants

Month of Delivery	Non-febrile Endometritis (Lochia Putrida)	Febrile Endometritis	All Puerperal Infections Combined	Pyrexia of Unknown Origin + Phlebitis	Infections of Mammary Gland	Pyelitis	All Urinary Infections during and after Delivery	Total No. of Deliveries
I—II	7 8	4 0	2 4	4	1 9	3 5	3 7	4278
III—IV	6 7	2 8	9 9	9 7	0-4	3 2	3 4	593
V—VI	6	2	8 3	9 9	8	5	8	4783
VII—VIII	6	4	8 9	9	9 7	2 5	2 7	4383
IX—X	5 4		7 9	8 0	8 3	3 0	3-3	4 19
XI—XII	5	9	8	7 8	8	3-2	3-4	3929
T tal	6 2	2 7	9 3	9	9 8	3-0	3-2	266 3

lation is more resistant to the stress of the winter but seems to be more susceptible to infections during the summer The present data throw no light on the reason for this phenomenon.

It is especially interesting to note that throughout Finland puerperal infections are much commoner following the birth of male rather than female infants. In every area studied and also in the country as a whole the sex ratio was higher in the infected group than in the control group. The phenomenon is probably connected with the fact that most delivery complications are more likely to occur with male infants than females (Timonen et al. 1963)

SUMMARY

1 Seasonal variations in obstetrical phenomena were examined in two series of deliveries. 27 522 cases from Helsinki (Series 1) and 57 089 cases from the whole of Finland (Series 2)

is probably connected with the phenomenon that the mortality of boys decreases more than that of girls during the summer (Pfaundler 1943, De Rudder 1952).

Neonatal Jaundice

Neonatal jaundice is a rather loose expression. In the absence of immunization it commonly results from excessive red blood cell destruction which occurs with the loss of the prenatal physiological polycythaemia consequent on improved oxygenization of the tissues. Clinically only very marked cases are included if jaundice is diagnosed visually. A highly significant seasonal variation can be seen (Table XI). There is no slowing effect towards the early summer. On the contrary, the decreased frequency is already evident in May–June. Jaundice is closely correlated with the oxygen saturation of the mother and of the utero-placental system in the latter months of pregnancy. It is not dependent on hypoxaemia arising suddenly during labour. It may be correlated with dietary factors, because deficiency of vitamin E has been found to promote the disruption of red blood cells (Nyhan, 1961). The fact that the frequency of jaundice decreases earlier than that of asphyxia shows that factors affecting the mother's blood circulation (elevated Hgb, more outdoor activity during the spring, varied diet, etc.) become normal earlier than a general readiness for a successful delivery.

Postpartum Infections

Infectious diseases were examined in both Series 1 and 2. A significant rise in the incidence of endometritis was revealed during January–February in Series 1. The cases of mastitis increased during the first half of the year, and pyelitis throughout the winter (Table XII). The summer and autumn can be regarded as the most favourable seasons in Helsinki. In the series comprising the whole country, however, the situation was the reverse (Table XII). The peak of infections occurred during the months VII–X. Such a clear geographical deviation must be based on a difference in mode of living between town and country dwellers. Even in this comparison it comes out clearly that the rural popu-

2. The conception rate was clearly higher in summertime than during the winter A delayed effect of light was observed in Series 2 (whole of Finland) as had previously been found in Series 1 (Helsinki)
3. The duration of pregnancy in Series 1 was slightly but significantly longer in summertime than in winter As a result of this phenomenon the incidence of prolonged pregnancy was increased. In Series 2 seasonal variations were evident only in South Finland. There were no differences in the incidence of prematurity
4. The total duration of labour decreased slightly in late summer at the same time the shortening of the second stage was highly significant in all groups.
5. Hyperemesis as estimated from the number of patients admitted to hospital was commonest during the winter and decreased in May
6. The lowest incidence of late pregnancy toxaemia was in July–August There was no actual peak.
7. The shape of the newborn was dependent on the month of birth in so far that the frequency of stout children was lowest in July–August. On the other hand, since it is known that children born in summertime are heavier the authors conclude that the gain in height of the foetus is greater in summertime
8. The sex ratio (boys/girls at birth) was lowest in pregnancies which commenced during June–July and December–January The authors attribute the former peak to the influence of climate The latter can be regarded as a secondary result of the high incidence of abortion in early spring.
9. There was a significant seasonal variation in asphyxiated newborn children from non-toxaemic pregnancies, the peak being in March–April.
10. There is a significant decrease in the cases of jaundice of the newborn in May June The variation is considered to be dependent on dietary factors
11. Obstetrical infections (endometritis, mastitis and pyelitis) were higher during the wintertime (Series 1) In Series 2 (whole of Finland) only endometritis and pyelitis were ex-

Table XIII *Seasonal frequency of post partum infections in series 2 The highest frequency of all infections combined is seen during months VII—X ($\chi^2 = 70$) Note the preponderance of male infants in cases with infection*

Month of Delivery	Helsinki		T + Tam		South		West		East		North		Whole country		Total No. of Deliveries	Frequency of p.p. Infections
	Total	Male Infants	Total	Male Infants	Total	Male Infants	Total	Male Infants	Total	Male Infants	Total	Male Infants	Total	Male Infants		
I—III	60	58 3	140	52 9	37	48 7	13	46 1	6	50 0	5	60 0	261	53 3	7681	3 4
III—IV	107	54 2	191	52 9	37	70 3	16	62 5	33	63 6	2	50 0	386	56 2	9483	4 1
V—VI	100	61 0	175	58 3	37	54 1	13	53 8	29	62 0	4	50 0	358	58 7	9031	4 0
VII—VIII	119	64 7	295	55 3	26	58 5	18	50 0	10	30 0	7	28 6	475	55 6	9499	5 0
IX—X	87	54 0	330	56 4	44	47 7	13	38 4	16	81 2	4	75 0	494	55 7	8637	5 7
XI—XII	82	46 3	151	57 0	37	56 8	13	53 8	10	30 0	5	100 0	298	53 7	7709	3 9
Total	555	56 9	1282	55 5	218	53 2	86	51 1	104	58 7	27	59 6	2272	55 7	52040	4 4

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amined. The peak occurred during months VII–VIII. The differences between urban and rural populations seems to depend on differences in living habits.

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Table I *Summary of the Clinical History of o Cases of Primary Ovarian Pregnancy*

Patient No	Anamnesis								
	Age	Parity	Abortions	Salpingo-oophoritis	Algo-menorrhea	Previous gynaecological Operations	Amenorrhea (weeks)	Nausea, Vomiting	Abdominal Pains (days)
1.	36	I	—	—	+	—	5	+	3
2.	25	II		—	—	—	6	+	
3	24	II	—	—	—	—	3	+	60
4	3	II		—	—	—	7	+	8
5.	3	—	—	—	—	—	7	—	
6.	23	—	—	—	—	—	3	+	1
7	20	I		—	—	—	3	+	4
8	36	VI	—	—	—	—	2		
9	24	I	—	—	—	—	5	+	3
10	22	—	—	—	—	Right ovarian resection	9	+	22
Mean	27						7		

Table II *Summary of the Clinical History of o Cases of Primary Ovarian Pregnancy*

Patient No	Uterine Bleeding	Shoulder pain	Signs of Shock	Abdominal Tenderness	Abdominal Tumours	Days in Hospital before Operation		>
		+	+	+	+	+		
		+	+	+	+	+		
3		—	—	—	+			+
4		+		+	+	+		
5	+		—	—	+		+	
6		+		+	+	+		
7		+	—		+	+		
8				+		+		
9		+	+	+	+	+		
					+			+

the fimbriated end, must be intact, and must be distinctly separate from the ovary 2) The gestational sac must definitely occupy the normal position of the ovary 3) The gestational sac must be connected to the uterus by the utero-ovarian ligament. 4) Ovarian

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PRIMARY OVARIAN PREGNANCY

BY

BERNDT JOHAN PROCOPÉ AND TUULIKKI VESANTO

Ovarian pregnancy is a rare type of ectopic gestation. The first valid case was reported by Saint Maurice of Perigod, France in 1862 (Ricci 1950) Böwing (1923) collected 100 cases from the literature, Kermauner (1932) 140 Stoeckel (1951) 200 Baden and Heins (1952) 100 Haselhorst (1953) 240 Moyers and Lack (1958) 200 and Fettig (1960) 240 cases.

The proportion of ovarian pregnancies in all ectopic gestations has been reported as 0 17–4 7 per cent (Säntti 1928 Parviainen 1943 Nucci 1946 Kinnunen and Jarvinen, 1952 Babrow and Winkelstein 1956 Ellis 1959 among others)

Compared with all pregnancies the ratios range from 1 25,000 to 1 40 000 (Baden and Heins 1952 Fettig 1960 Eastman and Hellman, 1962)

Ovarian pregnancy generally terminates during the first trimester (Baden and Heins 1952) but full term ovarian pregnancies have also been reported (Soikkeli, 1925 Säntti, 1928 Koskinen, 1950 Ullemeyer 1959 Hubacker 1963) Hydatidiform mole and eclampsia have been reported in connection with ovarian pregnancy (Wittenberg and Ries, 1948) Ovarian twin pregnancy has also been described (Green and West 1963)

The basic criteria for the diagnosis of true primary ovarian pregnancy were established in 1878 when Spiegelberg published the four postulates as follows 1) The tube including



Fig. Blood mole containing remnants of chorionic villi and trophoblast islets at the left. Wall of partly necrotized corpus luteum is at the right. Haem. + Gieson × 120.

tissue must be demonstrated in the walls of the sac. Norris (1909) further amplified the first postulate by stating that the tube must show no microscopic evidence of pregnancy.

Primary ovarian pregnancy may occur in two forms: Intrafollicular, where the fertilised ovum is implanted and develops in a Graafian follicle, and extrafollicular, where the fertilised ovum is implanted and develops in ovarian tissue outside a Graafian follicle.

Clinical Material and Results

The series consisted of 10 cases of primary ovarian pregnancy occurring during the years 1945–1963. A total of 2,327 ectopic pregnancies were seen during this time, and thus the proportion of ovarian pregnancies was 0.4 per cent. The results are shown in Tables I, II and III. Figs. 1 and 2 illustrate the intrafollicular manifestation.

The average age of the patients was 27 years. Seven gave a

Table III *Summary of the Clinical History of 10 Cases of Primary Ovarian Pregnancy*

		cm					
1	9.6	1000	Left 6 cm diam., cystic mass, ruptured	Intact	Ovarian parenchyma surrounding chorion villi. Intrafollicular	Ovarian resection	Uneventful
2.	9.8	500	Left 5 cm diam. cystic mass ruptured	″	″	″	″
3.	13.4	—	Right 8 cm diam. fœtus 1/2 cm long. Not ruptured	″	Ovarian parenchyma surrounding chorionic villi. Form impossible to define. Polycystic degeneration of other ovary	Oophorect. Right ovarian resection	″
4.	7.5	1000	Left 6 cm diam cystic mass ruptured	″	Ovarian parenchyma surrounding chorionic villi. Intrafollicular	Ovarian resection	″
5.	11.5	—	Right 4 cm diam., cystic mass, not ruptured	″	″		″
6	10.8	500	Right 1 cm diam. cystic mass, ruptured	″	Ovarian parenchyma surrounding chorionic villi. Form impossible to define		″
7	10.2	750	Right 5 cm diam., cystic mass, ruptured	″	Ovarian parenchyma surrounding chorionic villi. Intrafollicular	″	″
8.	11.1	500	Right 1 cm diam., cystic mass ruptured	″	″	″	″
9.	10.6	500	Right 5 cm diam., cystic mass ruptured	″	″	″	″
0	2.2	—	Right 6 cm diam., not ruptured. Fœtus 5 cm long	″	Ovarian parenchyma surrounding chorionic villi. Form impossibl [illegible]	″	″



Fig. Blood mole, containing remnants of chorionic villi and trophoblast islets at the left. Wall of partly necrotized corpus luteum is at the right. Haem.+ Gieson × 120

tissue must be demonstrated in the walls of the sac. Norris (1909) further amplified the first postulate by stating that the tube must show no microscopic evidence of pregnancy

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3.	13.4	—	Right 6 cm diam. foetus 1/2 cm long. Not ruptured	"	Ovarian parenchyma surrounding chorionic villi. Form impossible to define. Polycystic degeneration of other ovary	Oophorect. Right ovarian resection	"
4.	7.5	1000	Left 6 cm diam., cystic mass, ruptured	"	Ovarian parenchyma surrounding chorionic villi. Intrafollicular	Ovarian resection	"
5.	11.5	—	Right 4 cm diam., cystic mass not ruptured		"		"
6	10.8	500	Right 1 cm diam., cystic mass ruptured	"	Ovarian parenchyma surrounding chorionic villi. Form impossible to define	"	"
7	10.2	750	Right 5 cm diam. cystic mass, ruptured		Ovarian parenchyma surrounding chorionic villi. Intrafollicular		"
8	1 1	500	Right 1 cm diam. cystic mass ruptured	"	"	"	"
9	0.6	500	Right 5 cm diam., cystic mass ruptured	"	"	"	"
0.	12.2	—	Right 6 cm diam., not ruptured. Foetus 5 cm long	"	Ovarian parenchym surrounding chorionic villi. Form impossibl to define	"	"

either on the day of admission or on the following day The exceptions were patient No 10 who was in hospital for 2 days and patient No 3 who was in hospital for 10 days before operation. The operative procedure was ovarian resection except in Case 3 who was treated by oophorectomy At the same time unilateral ovarian resection for polycystic degeneration was done in Case 3. The postoperative course was uneventful in all the cases.

The ovarian pregnancy was of the intrafollicular type in 7 patients. It was impossible to define the origin accurately in 3 cases.

Discussion

There are several opinions concerning the pathogenesis of ovarian pregnancy Fertilisation could take place in the tube or peritoneal cavity then the ovum finds its way back and becomes implanted on the cortex or in the corpus luteum of the ovary A second possible mechanism is intraovarian fertilisation.

The intrafollicular form is generally regarded as a disturbance of ovulation itself *e.g.* because of the retention of the follicle in the vicinity of the rupture site (Höhne 1923 Haselhorst, 1953) difficulties in the discharge of the ovum when the hyaluronidase content of the ovum or granulosa cells is too scanty (Baden and Heins 1952) a very narrow rupture site or *vis a tergo* by the follicle fluid which should flood the ovum (Arronet, 1951)

The genesis of extrafollicular pregnancy is explained as an inhibited mechanism of discharge of the ovum due chiefly to mechanical factors such as inflammatory processes unevenness of the surface of the ovaries or hypoplastic tubes (Stingl, 1959) Not a single case with a history of salpingo-oophoritis was encountered in the present investigation. Nor was tubal hypoplasia seen. One patient had concurrent polycystic degeneration of one ovary One patient had undergone earlier resection of the affected ovary because of a benign cyst

Other authors give preference to the properties which the ovarian tissue must possess as the nidus for the ovum embedded in it. External endometriosis thus offers favourable soil (Sutton, 1924 Vara 1936 Courtis, 1941 McKenzie 1943 Kuzma



Fig. 2 Group of chorionic villi surrounded by partly necrotized ovarian tissue. Haem.+v Gieson. × 40

history of previous pregnancy Three patients had had an abortion. None of the patients had salpingo-oophoritis. Case No. 1 had been treated earlier for algomenorrhoea. Case No. 10 had undergone right ovarian resection because of a benign cyst. The average duration of amenorrhoea was 7 weeks the longest 13 and shortest 2 weeks. The clinical symptoms were typical of ectopic pregnancy Four patients showed signs of shock, 7 had a ruptured cystic mass in the ovary and free blood in the abdominal cavity It was possible to feel the foetus in 2 cases but in the other cases only parts of ovum were seen The patients were operated on

SUMMARY

Ten cases of primary ovarian pregnancy are presented. The average age was 27 years. All the pregnancies were terminated during the first trimester. The average duration of amenorrhea was 7 weeks. Seven of the patients had had an earlier pregnancy. The incidence was 0.4 per cent of all extrauterine pregnancies. Seven cases were of the intrafollicular type. Three cases were impossible to define more accurately.

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and Lillie, 1944 Norton and Alter 1945) This is probably a fairly rare cause of ovarian pregnancy Only two cases associated with ovarian endometriosis were encountered by Baden and Heins (1952) among 97 cases collected from the literature The rare occurrence of the condition was stressed also by Abrahamsen (1962) In the present investigation not a single case of ovarian or other external endometriosis was established.

According to Haselhorst (1953) the ratio of intrafollicular to extrafollicular sites is 100 10 But Miller (1937) and Novak (1947) claimed that the extrafollicular form is more common. Kermauner (1932) was of the opinion that it is always the intrafollicular form that is involved at first owing to its proteolytic activity it later grows through the wall of the follicle and the extrafollicular form originates secondarily A case of extra follicular ovarian pregnancy was reported by Simon (19 9) and Groot Wassink (1962) Böe (194-) reported one case but could not determine the form. The corpus luteum of pregnancy must have location other than the site of pregnancy in the extra follicular form either in the same or in the other ovary (Arronet, 1951) The present investigation included 7 intrafollicular forms. It was impossible to define more accurately in 3 cases All 10 pregnancies were terminated during the first trimester This concurs well with the findings of Svanberg (1949) Baden and Heins (1952) suggested that 75 per cent terminate during the first trimester while 25 per cent will last into the second trimester of which one half will last to the third trimester or beyond it. Approximately 66 per cent of the viable foetus will be stillborn and 18 2 per cent of these viable foetuses will be malformed. A foetus was found in 2 cases in the present investigation in the other cases only a cystic mass with chorion villi This shows that the ovum dies in an early phase or undergoes developmental disturbances. As there is more room in the wall of the ovary tubal pregnancy generally ruptures earlier

The average age was 27 years. Seven patients had had an earlier pregnancy Baden and Heins (1952) reported an average age of 30 years and 43 per cent with a previous pregnancy In the series reported by Reid and Vant (1963) the average age was 31 years and all the patients were multiparous.

SUMMARY

Ten cases of primary ovarian pregnancy are presented. The average age was 27 years. All the pregnancies were terminated during the first trimester. The average duration of amenorrhoea was 7 weeks. Seven of the patients had had an earlier pregnancy. The incidence was 0.4 per cent of all extrauterine pregnancies. Seven cases were of the intrafollicular type. Three cases were impossible to define more accurately.

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and Lillie 1944 Norton and Alter 1945) This is probably a fairly rare cause of ovarian pregnancy Only two cases associated with ovarian endometriosis were encountered by Baden and Heins (1952) among 97 cases collected from the literature. The rare occurrence of the condition was stressed also by Abrahamsen (1962) In the present investigation not a single case of ovarian or other external endometriosis was established.

According to Haselhorst (1953) the ratio of intrafollicular to extrafollicular sites is 100 10 But Miller (1937) and Novak (1947) claimed that the extrafollicular form is more common. Kermauner (1932) was of the opinion that it is always the intrafollicular form that is involved at first owing to its proteolytic activity it later grows through the wall of the follicle and the extrafollicular form originates secondarily A case of extrafollicular ovarian pregnancy was reported by Simon (1929) and Groot Wassink (1962) Böe (1942) reported one case but could not determine the form. The corpus luteum of pregnancy must have location other than the site of pregnancy in the extra follicular form either in the same or in the other ovary (Arronet, 1951) The present investigation included 7 intrafollicular forms. It was impossible to define more accurately in 3 cases All 10 pregnancies were terminated during the first trimester This concurs well with the findings of Svanberg (1949) Baden and Heins (1952) suggested that 75 per cent terminate during the first trimester while 25 per cent will last into the second trimester of which one half will last to the third trimester or beyond it. Approximately 66 per cent of the viable foetus will be stillborn and 18.2 per cent of these viable foetuses will be malformed. A foetus was found in 2 cases in the present investigation in the other cases only a cystic mass with chorion villi This shows that the ovum dies in an early phase or undergoes developmental disturbances. As there is more room in the wall of the ovary tubal pregnancy generally ruptures earlier

The average age was 27 years. Seven patients had had an earlier pregnancy Baden and Heins (1952) reported an average age of 30 years and 43 per cent with a previous pregnancy In the series reported by Reid and Vant (1963) the average age was 31 years and all the patients were multiparous.

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SPLIT PELVIS AND DELIVERY

Report of two deliveries in woman with split pelvis without ectopia vesicae

BY

SIMO LAURILA

An anomaly of the bony pelvis in which the pubic bones are not joined by fibrocartilaginous tissue but are widely separated is referred to as pelvis fissa. The English term is split pelvis and the German "Spaltbecken This congenital anomaly almost invariably occurs in association with exstrophy of the bladder and only rarely without it Fortunately exstrophy of the bladder is rare occurring only once in about 50,000 deliveries (Neudorfer) or according to some other statistics slightly more often It is four to five times as frequent in men as in women.

In bladder exstrophy the anterior wall of the bladder usually is absent, and the posterior bladder wall with the ureteric orifices is visible This lesion is severely incapacitating, distressing and dangerous. There is dribbling or spouting of urine so that the patient is constantly wet and the kidneys are prone to diseases such as infections, renal calculi, hydronephrosis etc. Until surgical treatment with uretero-colic anastomosis became widely used and effective drugs against pyelonephritis, e g sulphonamides and many antibiotics, became available these patients generally died young According to Lotimer (954) 50 per cent of untreated patients with ectopia vesicae die before reaching 10 years of age and only one third of them pass the 20th year of life

Women not treated surgically for ectopia vesicae experience difficulties in sexual intercourse for obvious reasons but their fertility is often good. Indeed, deliveries of these untreated

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bones has been demonstrated (Waldstein 1897 Meyer 1925 Goecke 1932 Clemetson 1958) but the ligament can be present only in anomalies of first or second degree. The rectus abdominis muscles are divaricated and inserted into the separated bones. This triangular window encloses the more or less malformed bladder The umbilicus is situated at a lower level than normal or is absent. The rare condition of a patent urachus has also been described in which case urine escapes from the umbilicus. Umbilical hernia occurs frequently The external genitalia and the vaginal introitus are often situated more ventrally than normal between the pubic bones. The labia are usually far apart and may be incomplete The clitoris may be absent it may also be large or cleft. Uterovaginal prolapse is common after vaginal delivery and even virginal prolapse has been described in these patients. For this reason the cervix is often visible in the introitus and the vagina is short. Inguinal herniae are frequently present. The iliac bones and both sides of the bony pelvis have drawn away from each other by rotating laterally around the sacro-iliac joints. Thus the acetabuli are farther apart than usual the perineum is broad, and the medial surfaces of the thighs are widely separated. The pelvic canal is generally large with a square cross section (pelvis quadrata) and the external conjugate if measurable is normal or greater than normal. Breus and Kolisko (1900) reported the results of their thorough investigation of the anatomy of split pelvis They distinguished two main types chiefly on the basis of the anatomical structure of the sacrum. In type I the anterior surface of the sacrum is straight or convex and the coccyx is usually turned towards the pelvic canal. In type II the sacrum has a concave essentially normal anterior surface The third main type of split pelvis is intermediate between types I and II The case to be reported below represents Breus and Kolisko s type I

Exstrophy of the bladder and split pelvis are often associated with deformities other than those stated above. As far as is known Creve (1795) was the first to call attention to the fact that other developmental anomalies of the urogenital tract occur in connection with ectopia vesicae such as anomalies due to incomplete fusion of the lower parts of the Müllerian ducts *e.g*

patients with ectopia vesicæ have been described in a number of reports

While split pelvis almost always occurs as a congenital anomaly in connection with exstrophy of the bladder a few cases have been described of split pelvis without bladder anomaly in women with no history of trauma that might account for absence of the symphysis (Kouwer 1900 Goecke 1932 MacKenzie 1935 Fuhrmann 1959) The case reported here also belongs to this group The case described by Kouwer is peculiar in that split pelvis occurred without bladder exstrophy but the skin over the bladder showed a strip of bladder mucosa Breus and Kolisko (1900) in their extensive work on anomalies of the pelvis took the position that congenital split pelvis never occurs in the absence of ectopia vesicæ and that the reported cases are due to trauma (Waldstein 1897 Muret, 1903) this view has had much support especially in the German literature (Meyer 1925 Damm 1937) in spite of the increasing number of cases reported without known traumatic aetiology In recent years a number of authors e.g Clemetson (1958) who reviewed the literature extensively and found 64 pregnancies in patients with split pelvis, have expressed the opinion that split pelvis may occur congenitally without other splitting phenomena of the anterior abdominal wall Clemetson accepts Champneys grading of ectopia vesicæ (1877) into four degrees

First degree The pubic bones are separated but there is no fissure in the muscles and fasciæ through which the bladder can protrude

Second degree The bladder is perfect but protrudes in the midline between the pubic bones and the rectus abdominis muscles.

Third and fourth degree The pubic bones are separated the bladder wall and abdominal wall are cleft.

The term split pelvis is used in practice to indicate absence of the symphysis pubis In theory the pelvic ring may be split in any other place but such cases are extremely infrequent —The distance between the pubic bones varies from a few centimetres to 14 9 cm. the maximum recorded in the literature I have seen (Freund 1872) In several cases a ligament uniting the pubic

puberty she had been suffering from incontinence of urine of second degree The family history revealed nothing of note.

Menstruation commenced at 15 years and from the age of 17 there had been a regular 3–4 days loss every 28 days. She married in January 1962. She had no abortions. The expected date of confinement for her first pregnancy was July 5, 962. In July 962 she was treated at a small hospital and because of suspected postmaturity she was given intramuscular syntho-cinon injections on July 18 and 1 but labour did not ensue. Spontaneous labour began on July 28 and after strong labour of 8 hours a female child was delivered, in left occipito-anterior position, birth weight 3,650 gm. The second stage lasted 25 minutes and the delivery seemed to be normal but the child was severely asphyxiated and lived only 5 minutes Because of copious bleeding during the third stage the placenta was removed manually

The patient's pelvic anomaly escaped notice during this delivery

The patient soon became pregnant again (last menstruation January 28, 963) During pregnancy she felt well, there were no signs of toxaemia. The Wassermann reaction was negative Rhesus group positive and Coombs test negative. The expected date of confinement was November 5, 1963.

On admission on November 9, 1963 the patient had weak irregular contractions. The blood pressure was 05/70 there was no proteinuria or oedema. The foetus lay in left occipito-anterior position, the circumference of the abdomen was 97 cm and the fundus uteri was 3 cm. below the xiphoid cartilage. The pelvis appeared capacious, the interspinous diameter was 28 cm. and the intertrochanteric diameter 34 cm. The external conjugate diameter was difficult to measure as there was no symphysis. The pubic bones were felt far apart and the foetal head protruded forwards in the area corresponding to the symphysis beneath the thin abdominal wall The perineum was broad and the labia were widely separated The clitoris was of normal appearance.— X-ray confirmed the lie of the foetus there was an 8 cm. distance between the pubic bones, and the cross-section of the pelvis was very large The conjugata vera was estimated t cm the anterior surface of the sacrum was slightly convex (Figs and b) According to skeletal development the foetus was at term.

On November 20 at 8 m the patient went into labour the pains occurring every four to five minutes Vaginal examination two hours later showed the cervix 3 cm dilated and vertex presenting at station −2. The membranes were ruptured and clear amniotic fluid escaped but the delivery did not proceed well At 5 p m an intra enous syntocinon infusion was started (containing 5 units syntocinon in 500 ml of 5 % glucose solution) and the deli ery then proceeded without any signs of asphyxia of the foetus. At 3 5 p m the cervix was 6 cm dilated The second stage began at 4.40 p.m. and 5 minutes later the patient was delivered of viable female child weighing 4,250 gm A medium sized episiotomy was made and there was no perineal tear Blood loss during the third stage was 700 gm but bleeding

vaginal septa uterus unicornis uterus bicornis and uterus duplex or ectopic and abnormally shaped kidneys, double ureters etc Other associated anomalies are spina bifida cleft palate hydrocephalus etc. Ectopia vesicæ and split pelvis are not hereditary conditions No cases have been described of this anomaly occurring in two or more successive generations. Schickele (1901) reported a woman with ectopia vesicæ whose dead sister had had "deformed genitalia" and Randall (1934) a woman with ectopia vesicæ whose two brothers, who died young, had also had bladder exstrophy

Deliveries in women with split pelvis have been rare for several obvious reasons For one thing this anomaly of the bony pelvis in itself rare is nearly always associated with exstrophy of the bladder which before the days of surgical treatment and modern antibacterial therapy decisively shortened the patient's life Also difficulties in sexual intercourse prevented these women from becoming pregnant. Today thanks to successful surgical treatment of ectopia vesicæ an increasing number of affected women reach adult life and can marry which means that deliveries in these cases will also increase Indeed several isolated cases and small series dealing with deliveries of patients previously treated by uretero-colic anastomosis for repair of ectopia vesicæ have been reported in recent years (Randall 1934 MacKenzie 1935 Lotimer 1954)

The purpose of this paper is to report a personally encountered case of split pelvis without evidence of any associated splitting of the anterior abdominal wall and two deliveries of this woman and finally to discuss the special obstetric problems of these women in the light of previous reports

Case Report

The patient, a secundigravida aged 25 was first admitted to the Obstetric Department of the Central Hospital of Southern Saimaa on November 19, 1963, for her second delivery The past medical history was uneventful, rachitis had never been diagnosed and the patient had never suffered an accident of any importance. She had never had any pain in the pelvic region. In her school days she took her normal place in sporting activities. From



Fig. b

Another striking feature was the marked backward curvature of the knee joints (Fig. 4) but other joints showed no distinct hyperflexibility. On cystoscopy the bladder was found to hold 350 ccm. without difficulty. The bladder and the ureteric orifices were normal. Intravenous urography showed kidneys with normal location size shape and excretion. Urethrocystography showed



Fig 1 a

then ceased. The puerperium was entirely uncomplicated. The patient had no pelvic pain following the delivery

The laboratory findings were as follows haemoglobin 13.4 gm per 100 ml, haematocrit 42 % mean corpuscular haemoglobin concentration 32, leucocyte count 16 500, serum calcium 4.5 mEq/l, serum proteins 7.3 % acid phosphatase 0.23 Bessey-Lowry units, alkaline phosphatase 2.0 Bessey Lowry units. The serum protein electrophoresis was normal.

At postpartum clinical examinations attention was aroused especially by the marked mobility of the pelvic bones. As the pubic bones could be felt beneath the skin about 9 cm apart it was easy to force them together by applying pressure on the sides of the pelvis the movement occurred at the sacro-iliac joints.



Fig. 6

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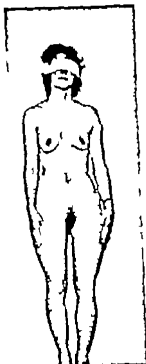


Fig 3

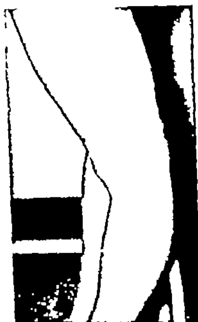


Fig 4

Discussion

A number of papers have been published dealing with cases described earlier in the literature (Randall 1934 Lotimer 1954 Clemetson 1958) With rare exceptions, split pelvis is associated with the congenital deformity known as bladder exstrophy Clemetson (1958) made a thorough search of the literature and found 64 recorded cases of pregnancies in 45 women with split pelvis. Fuhrmann (959) has since described one delivery of a woman with this bony anomaly without exstrophy of the bladder All these analyses indicate that the pregnancies and deliveries of these patients often present various irregularities. In Clemetsons series, the lie of the fœtus was known in 40 cases, and 15 of these were malpresentations e g breech, transverse and compound presentations. This is evidently due to the



Fig 2

slight descent of the floor of the bladder but no other evidence of pathology Hysterosalpingography showed uterus arcuatus. The apparently large eyes of the patient prompted ophthalmological examination too The *only positive finding was that the bulbi* were found to be remarkably large without noticeable exophthalmos (Hertel 16–100–16 mm.) and the scleræ were bluish and apparently thin The patient was kept under observation until July 1964 by that time (Fig 3) the separation of the pubic bones had diminished to 5 cm (Fig 2) and a firm ligament was felt between them. Apart from slight cystocœle there was no other uterovaginal prolapse For some unexplained reason the patient, following her second delivery had no symptoms of urinary incontinence which she had suffered from previously for 10 years

does occur in virgins or may commence during pregnancy (Ismail, 1939 Clayton 1945 Hinman 1951) A number of authors, e g Randall and Lotimer hold the view that a uretero-sigmoid anastomosis does not argue against vaginal delivery but the anal sphincter in particular must be protected from obstetric trauma and a wide episiotomy is indicated. There is some knowledge of deliveries in cases of bladder exstrophy where an urethra is present and where after surgical closure of the bladder there is continence of urine Some pregnancies of these patients were terminated by abdominal section (Sjövall) but at least one patient was delivered vaginally without trouble (Clemetson) There is no evidence of hereditary tendencies and hence sterilization is not indicated at least not for eugenic reasons.

SUMMARY

It is evident that deliveries in cases of split pelvis are increasing, due in the main to successful surgical treatment for bladder exstrophy A woman with split pelvis is reported, with a description of her two deliveries. A number of characteristic features in deliveries of women with split pelvis are discussed, e g the high incidence of malpresentations, premature rupture of membranes and prematurity stillbirths and tendency to uterovaginal prolapse after delivery Hereditary tendencies have not been demonstrated.

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fact that a wide pelvic inlet fails to control the presenting part, and also to the frequency of uterine anomalies in these patients. Premature deliveries seem to occur especially in women who also have bladder exstrophy Von Frangue and Damm have stated that 33 per cent of the foetuses are born prematurely and this surely contributes to increase the tendency to malpresentation Premature rupture of the membranes generally marks the onset of labour Each of the two pregnancies here reported was probably past term

The high percentage of stillbirths is a characteristic feature in these cases. Clemetson's series included 9 cases of intrauterine death just preceding or during labour and in addition 3 babies were lost immediately after delivery This may be explained in part by difficult deliveries malpresentation prematurity etc., but very often reports have stated that the baby has died *in utero* without apparent cause just about the time of onset of labour (Randall 1934 Damm, 1937 Goecke 1932) In the first delivery of the present patient the baby was also lost, which in this case is accounted possibly by postmaturity

Patients with split pelvis have a marked tendency to utero-vaginal prolapse According to Clemetson uterine prolapse was known to have developed later in 20 out of 34 patients with bladder exstrophy delivered vaginally in 7 women delivered of 8 infants by abdominal section the subsequent progress was known and in none of them did prolapse occur

The extreme variations observed in the management of deliveries in cases of split pelvis makes classification difficult The vast majority of these deliveries have been vaginal and have required various auxiliary incisions in the external genitalia. Vaginal and abdominal Caesarean sections of many kinds have also been performed The marked tendency for uterovaginal prolapse to develop especially in the case of third and fourth degree ectopia vesicae argues against vaginal delivery the more so as the surgical repair of prolapse in these patients presents great difficulties. However in 4 women with *split pelvis without* bladder exstrophy delivered vaginally of 5 infants prolapse did not subsequently develop (*Kouwer 1900* Clemetson 1958 Fuhrmann 1959 and the present case) It is known in addition that prolapse

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OBSTETRIC STUDIES IN WOMEN WITH CONGENITAL SOLITARY KIDNEYS

BY

CARL FELDING

In the literature congenital solitary kidney (CSK) is considered alternatively as either the result of renal agenesis or aplasia. It is not possible to make this distinction clinically as neither radiological nor operative findings are necessarily conclusive

The symptomatology of congenital solitary kidney depends upon the function of the existing kidney and the possible coexistence of other malformations. The anomaly is of great obstetric interest. The condition is frequently symptomless and is then only diagnosed at routine autopsy CSK alone or combined with malformations of the genitalia is however responsible for complications during pregnancy and delivery so that the obstetrician treating the case has the opportunity for establishing the correct diagnosis more frequently than is the case at present.

From an obstetric point of view the following questions concerning CSK are of the greatest importance 1 incidence 2. sex distribution, 3. combination with malformations of the female genitalia and 4 function of the existing kidney

1 Incidence Ekström (955) states, from a review of autopsy reports in the literature that the incidence of unilateral agenesis of the kidney is as follows

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e g with malformation of the calyces (Ekström, 1955) Ogilvie (1957) reported a case of pregnancy in a patient with a uterus unicornis and renal aplasia with reduced function of the solitary kidney The patient died from uraemia one year after therapeutic abortion. Goodlin (1959) described renal insufficiency during pregnancy as shown by acidosis and isostenuria

The author s material

As part of a study concerning the course of delivery in women with histories of renal disease 13 patients with congenital solitary kidney are presented. In these patients the course of all the pregnancies is known and data are available concerning the situation of the kidney Nine cases originate from the Surgical Obstetrical and Medical Departments The General Hospital in Malmö and four from the Department of Obstetrics and Gynaecology in Lund and Jönköping These figures give no indication of the incidence in medical, surgical or obstetric units as patients with asymptomatic CSK, ignorant of their condition must have passed undiagnosed through the various departments.

The data are tabulated in Fig. 1 The summary shows that these 13 women had 39 pregnancies which resulted in 17 living infants. Further it is found that the diagnosis of CSK was not established until after a long and unsatisfactory obstetric history The combination with genital malformations was present in at least 9 out of 13 cases. Two out of the 4 women about whom no definite information was available concerning genitalia did, however show obstetric complications suggestive of genital malformation (premature labour abortion)

The solitary kidney is frequently damaged in some way This damage may be either congenital or acquired. In this limited series damage was present in three out of 3 cases.

In 11 cases the diagnosis was established late in pregnancy or after delivery and the obstetricians concerned thus had no opportunity for prophylactic investigation and treatment prior to or during the pregnancies.

In two cases, the diagnosis of congenital solitary kidney was established already prior to the pregnancy These two cases will be briefly summarized

Author	Date	Incidence	Number of Autopsies
Anders	1910	1 1817	92,690
Eisendrath	1924	1 1000	77,812
Fortuna	1927	1 1290	139,346
Campbell	19-8	1 1444	13,000
Campbell	1928	1 1610	122,230
Collins	1932	1 920	337,488
Eisendrath & Rolnick	1938	1 953	159,274
Nation	1944	1 1930	27,000
Bell	1950	1 615	59,064

The average incidence is thus 1 1275.

2 Sex distribution Nation (1944) in 27 cases found 18 males and nine females Collins (1931) found 55 per cent males in 512 autopsies

3 Combination with malformations of the genitalia Ekström (1955) in 24 cases of renal aplasia found four with co-existing genital anomalies with uterine and/or vaginal duplication Schumacher (1938) treated the problem on the basis of congenital anomalies of the genitalia He found a high incidence of genital anomalies among patients with CSK this incidence being higher for females than for males. Ogilvie (1957) found in the literature 39 cases of CSK among 53 cases of uterus unicornis. Semmens (1962) presented 91 cases of genital malformations from an extensive series which had been submitted to urography In this material there were 31 cases of CSK or about 42 per cent. Semmens emphasized further the serious obstetric prognosis in all cases of uterine malformation with a fœtal mortality of approximately 49 per cent. Collins (1931) found among 231 women with CSK 90 per cent with concomitant malformations in genitalia Woolf and Allen (1953) described the records of 35 patients with various genital malformations. They found among 15 cases with one Müllerian duct imperfectly developed 12 cases of CSK

4 According to Gutierrez (1933) the solitary kidney is frequently the site of stone formation hydronephrosis, pyelonephritis and nephritis In addition it may frequently be deformed

1. N. born in 1943. The diagnosis of congenital solitary kidney i.e. absence of the right kidney was established elsewhere in connection with investigation for recurrent urinary infection. Gynaecological investigation revealed single vagina and double uterus, the left part being normal and the right rudimentary and communicating with the left at the cervical canal. In November 963, salpingectomy was performed on the right side with the object of preventing nidation of a fertilized ovum in the rudimentary right uterus. During investigation, diabetic heredity was revealed and the result of the glucose tolerance test was abnormally high although the fasting blood sugar was normal. There were no signs of renal insufficiency. The patient became pregnant after the last menstrual period in February 964. She was admitted between the second and third months for prophylaxis against abortion and for planning of future treatment. In May 964, threatened abortion occurred with haemorrhage, pyrexia and suspected leakage of amniotic fluid. This diminished during prolonged stay in hospital and administration of antibiotics etc. In August 964, a urinary infection was successfully treated with chemotherapy. Caesarean section was undertaken at term on the indications of breech presentation in a primigravida with malformation of the uterus. The infant weighed 3690 g (8 lb) and was normal.

J born 934. The diagnosis of double uterus and double cervix was established in 96 when the patient was submitted to operation for removal of right-sided dermoid cyst, right salpingooophorectomy being undertaken at the time. The patient attended hospital in 963 on account of sterility. The investigations undertaken showed among other things, absence of the right kidney with compensatory enlargement of the left kidney. Further a half extra vertebral body was present between L 5 and S. The patient first pregnancy ended with abortion in October 1963. During the subsequent pregnancy with the last menstrual period in December 963, the patient was repeatedly admitted for prophylaxis against abortion. A glucose tolerance test in May 1964 showed normal findings. Caesarean section was undertaken at term on the indication of breech presentation in multipara with malformation of the uterus and prolonged sterility. The infant was boy weighing 3830 g (8 lb 7 oz) and was normal. The puerperium was uneventful.

Similar experiences have been described by Bret and Legros (962) and Magnin *et al* (1960).

Hysterosalpingography should be undertaken in all fertile women with congenital solitary kidney and urography in all patients with genital malformations.

In cases of unsuccessful pregnancies with recurrent abortions, premature deliveries, atypical toxaemias of pregnancy and protracted urinary symptoms congenital solitary kidney and uterine malformation should be suspected.

	A b. 1915	H 1916	A 1919	N 1926	O 1911	K 1933	A 1928	O 1922	E 1937	L 1931	N 1943	E 1937	J 1934	TO
ABORTIONS PREGNANCIES EXTRAUTERINE	0	0	2 THERAPEUTIC 2 SPONTANEOUS 4	0	[illegible] SPONTANEOUS 1 THERAPEUTIC 1 X 5	2	0	0	1	2	0	3	1	1
INFANTS <2500	0	0	0	1	3	0	0	0	1 CAESAREAN SECTION	0	0	2	0	7
INFANTS 2500	2	1 BREECH †	1	2	0	2	1 BREECH CAESAREAN SECTION	1	0	2	1 BREECH CAESAREAN SECTION	0	1 BREECH CAESAREAN SECTION	14
TOTAL NO OF PREGNANCIES	2	1	5	3	8	4	1	1	2	4	1	5	2	39
LIVING INFANTS	2	0	1	3	2	2	1	1	1	2	1	0	1	17

Fig 1

L'auteur base sa documentation sur 13 cas de malades à rein congénital unique. Ces patientes ont été enceintes 39 fois. 18 gravidités ont pris fin pendant le premier ou le deuxième trimestre (avortements et grossesses extra-utérines) 21 ont été menées à viabilité. En tout, le nombre de naissances d'enfants vivants est de 17 alors que le total des pertes fœtales est 22 sur 39 c. à d. 56 %. *L'intervention césarienne y est très fréquente.*

L'hystéro-salpingographie est conseillée pour toutes les femmes fertiles porteuses d'un rein congénital unique, et l'urographie pour toutes celles ayant des malformations génitales.

Acknowledgement

The author takes this opportunity of expressing his gratitude for access to the case histories to Professor J Waldenström of the Department of Medicine in Malmö to Professor A. Sjövall and G Widlund M. D., of the Departments of Obstetrics and Gynæcology in Lund and Jönköping, respectively

After having finished this article another case of CSK has been observed (aplasia renis sin.) This woman has 4 living children and no miscarriage The first infant had a birth weight of 1730 g, the others more than 2500 g

Including this case the total series thus comprises 14 patients with 43 pregnancies. Twenty-one infants survived. Total fœtal loss 22 of 43 = 49 per cent.

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SUMMARY

Congenital solitary kidney is a condition which occurs with an incidence of approximately 1 1200 in the overall population On account of simultaneous malformations of the genitalia, patients with this condition are of great obstetric interest as malformations of the uterus may cause abortions premature deliveries abnormal presentations etc Further defective function of the solitary kidney may exert an unfavourable effect upon pregnancy and delivery

The condition is frequently undiagnosed *in vivo* but may be revealed for example *in connection with complications of delivery* resulting from associated malformations of the uterus.

The author s series consists of 13 patients with congenital solitary kidney and in these women a total of 39 pregnancies occurred. Eighteen pregnancies terminated in the first and second trimesters (abortions and extrauterine pregnancies) and 21 went to term A total of 17 living infants were delivered and the total fœtal loss was thus 22 out of 39 = 56 per cent The incidence of Cæsarean sections was high

Hysterosalpingography is recommended in all fertile women with congenital solitary kidney and urography in all cases with malformations of the genitalia

Resumé

L anomalie appelée rein congénital unique se présente à une *fréquence estimée de 1 1200 dans une population normale* A cause des malformations génitales qui y sont associées les malades sont des cas *intéressants* au point de vue *obstétrical puisque les* malformations utérines peuvent avoir comme conséquence des complications, *telles* que *fausses couches accouchements* avant terme présentations anormales du fœtus etc En outre un mauvais fonctionnement du rein *unique* peut agir *défavorablement* sur la grossesse et l accouchement.

Ces cas évoluent le *plus souvent non-diagnostiqués in vivo* mais peuvent être découverts par exemple conjointement à des complications d accouchement *où l on peut constater que les* malformations utérines sont la cause même de ces complications.

L'auteur base sa documentation sur 13 cas de malades à rein congénital unique. Ces patientes ont été enceintes 39 fois. 18 gravidités ont pris fin pendant le premier ou le deuxième trimestre (avortements et grossesses extra-utérines) 21 ont été menées à viabilité En tout, le nombre de naissances d'enfants vivants est de 17 alors que le total des pertes foetales est 22 sur 39 c. à d. 56 %. L'intervention césarienne y est très fréquente.

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IMPETIGO HERPETIFORMIS — A CASE IN A PREGNANT WOMAN TREATED WITH ANTEX

BY

KAI AXEL RASMUSSEN AND MARGRETHE-L. EHRENSKJÖLD

Using the term impetigo herpetiformis Hebra in 1872 described a disease which he had observed in five pregnant or puerperal women. The condition manifested itself partly as extensive skin lesions the initial characteristic being groups of pustules and partly as severe systemic disturbance. Four of the five patients died.

In 1893, Kaposi stated that the disease, including Hebra's cases, had been diagnosed at the dermatological clinic of the Vienna University in 15 women all of them late in pregnancy or in the puerperium. In the meantime Kaposi (1887) had also seen a male patient, and since then the disease has been observed several times both in men and in non-pregnant women. Bruhns, in a critical survey of the publications up to 1925, found that 5 cases in males one of them an 11 year-old boy and 3 cases in non-pregnant females were true cases of the disease. In the literature from 1921 to 1958 Möslein (1959) found 113 presumably true cases with the following distribution pregnant women 54 per cent, non-pregnant women 36 per cent, and men 10 per cent.

On the basis of the first statements, the disease must be regarded as a most serious one thus, 14 of Hebra's and Kaposi's first 16 patients died, and Glaevecke found a mortality of 80 per cent among the 20 cases described up to 1896. Eventually reports of many cured cases appeared of 67 patients between

1921 and 1958 according to Mösleln (1959) 17 died *i e* 25 per cent.

The ætiology is unknown As mentioned the disease is not confined to pregnancy The pustules have been sterile or have contained casual bacteria In 1921 Schardorn published two cases of impetigo herpetiformis in the course of tetany due to *hypoparathyroidism and since then, hypocalcæmia* or tetany has been reported in nearly half of the cases (Mösleln 1959)

Formerly the diagnosis was based solely on clinical criteria. Kogoj found in 1938 and 1951 that the histological structure of the pustule in impetigo herpetiformis was identical with the one which he had described in 1927 as the spongiform pustule in acrodermatitis continua of Hallopeau and which he also had found characteristic of pustular psoriasis of Zumbusch s type Several authors (Arrighi 1962 Gaumond 1956 Hadida and Timsit 1956 Huriez *et al.* 1962 Lapiere 1958 Leonhardi and Michel, 1958 Manchester 1960 and Mösleln 1959) who have communicated histological examina tion of their cases of impetigo herpetiformis during recent years describe this form of pustule These microscopic findings must, although unspecific be considered so typical of impetigo herpeti formis as to be a *conditio sine qua non.* As a result of these findings one should be able to exclude diseases with which impetigo herpetiformis may have been confused such as herpes gestationis, dermatitis herpetiformis (especially its pustular form) and the subcorneal pustular dermatosis of Sneddon and Wilkin son However the histology will be of no differential diagnostic significance in relation to psoriasis pustulosa of Zumbusch the presence or the absence of ordinary psoriatic lesions must be considered as the only evidence which will permit us to decide whether we are faced with a case of psoriasis pustulosa or impetigo herpetiformis.

The treatment must be directed at correction of the hypopara thyroidism if this be present Of the many other agents which have been tried the gonadotrophic hormone from serum of preg nant mares Antex appears remarkable Tenlén used this pre paration successfully in a pregnant woman in 1937 Heller ström and Westman (1945) have reported on its good effect

in another pregnant woman, and in Tenlén's patient during two later pregnancies. This patient who, following termination of pregnancy and salpingectomy had a severe recurrence was also treated with Antex by Thyreason (1948). He observed repeatedly a distinct correlation between the amount of the dose and the symptoms and emphasized the importance of large doses for a long time. Engfeldt and Gentele (1950) did not see any effect of Antex in a non-pregnant woman and Hvidberg (1957) reported on a good result in a case which occurred and was treated during the puerperium, but a negative result in a woman who had recurrences at the time of her menses after the disease had started during a pregnancy 12 years earlier.

Report of Case

The patient is an 18-year-old woman. There is no history of psoriasis in her family. As child she was said to have had eczema on her trunk and scalp but had no skin disease for the last ten years, and she was otherwise healthy. The menarche began at sixteen years. There was no previous pregnancy. Her last menstruation was Oct. 30 962. Hyperemesis occurred in the third month of pregnancy with loss of weight (8 kg). After the administration of fluids (Med. Dept. Kolding Sygehus, Jan. 7–22, 963) she recovered and regained her normal weight.

The patient was first seen on April 30, 963. She was 27 weeks pregnant. Over period of 3 weeks groups of to 4 miliary tense pustules with a very red base had developed on the abdomen. A few groups had coalesced to form oval scale-crust-covered plaques. There was no itching. The patient looked pale and little tired. She was afebrile. The condition was diagnosed as impetigo herpetiformis. The treatment was initiated with Avlosulfon, 00 mg twice daily and two days afterwards supplemented with Ledermycin, 50 mg four times daily. During the next six days new lesions were seen to appear with increasing speed, and the patient health generally got worse with tiredness, dizziness nausea and vomiting. Her face was greyish in colour and the pulse-rate increased to about 00.

She was admitted to the Department of Obstetrics and Gynaecology Kolding Sygehus on May 6 with the lesions involving extensive areas (Figs. and 2). The abdominal, genital and inguinal regions were affected as whole; on the rest of the trunk there were numerous plaques one hand-sized on the breast- and the extremities were covered with great many small efflorescences. It seemed as if three stages could be distinguished) the initial groups of to 4 miliary pustules surrounded by an erythematous halo measuring 5– cm,) oval, red plaques covered with grey-brown scale-crusts measuring 4 to 5 cm (formed by confluence of the

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Fig. On admission. Numerous small groups of pustules surrounded by erythema, partly scattered and partly coalesced into larger plaques.

I.U. daily. In the following 6 days few efflorescences arose, but all the elements present grew larger and more and more of the lesions coalesced. When scale-crusts were removed a slightly moist surface was uncovered in the depth of which numerous miminal pustules could be suspected. A strong smarting sensation in the skin hampered the movements of the trunk. There was almost continuous vomiting. Liquids were administered parenterally. The complexion was alarmingly pale and greyish, and the lips were cyanotic. The patient was ailing and apathetic. Avlosulfon and Ledermycin were stopped after having been given for 3 and 1 days respectively. The dose of Antex was increased to 500 I.U. daily. Two days later the erythema began to fade, the movements of the trunk became freer and the general condition was considerably improved, vomiting ceased, the appetite returned, the colour of the face became normal and the cyanosis of the lips disappeared. In the following days, the skin lesions were

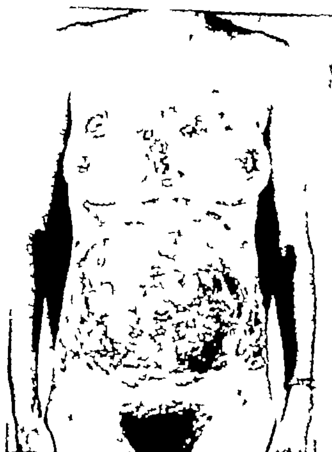


Fig. 1 On admission. Extensive erythematous areas covered with scale-crusts.

initial groups) and 3) very extensive areas of the same appearance with curved borders arising from a confluence of the secondary plaques Furthermore a number of red flat papules a few mm broad, had appeared on the soles of the feet. Afterwards, similar elements appeared on the palms

A biopsy from the scapular region was performed on May 7 and the histological examination was carried out by *Fridtjof Bang*, M.D (Fig. 3) The epidermis was thickened. It contained parakeratotic layers in its upper part. The rete pegs were short and rounded. Between the layers of parakeratosis in the uppermost stratum malpighii a Kogoj pustule was seen. It contained numerous neutrophils. In the marginal areas it was seen that it came into existence by the following process The epithelial cells were swollen and the nuclei were perishing while the cellular walls were preserved and, at the same time neutrophils were invading. In the dermis many capillaries were dilated and surrounded by small infiltrates of leukocytes and lymphocytes.

Immediately after admission, treatment was initiated with Antex, 750

100/min, for the next two weeks about 90, and for the next two weeks about 80. After withdrawal of Antex and until delivery the pulse-rate was 90 to 00 post partum it was between 60 to 80

Other examinations Height 167 cm weight 59 to 63 kg Rh positive. Serum calcium. 9.0–9.1–8.4–9.2–9.4–9.5–9.3 mg/ 00 ml. Serum phosphate 3.5–4.2–3.7 mg/ 00 ml. Urine calcium. 9.4– 3.7 mg/ 00 ml. Urine phosphate. 659–91.5 mg/ 00 ml. Acid phosphatase 4.0 mg/ 00 ml. Alkaline phosphatase 7.5 mg/ 00 ml. Serum sodium, potassium, chloride, carbon dioxide proteins all normal. Serologic tests for syphilis (Wassermann and Kahn) were non-reactive. No proteinuria nor glycosuria. Microscopic analysis of the urine was normal. Haemoglobin between 64 and 99 %. Blood counts were normal, no eosinophilia. Chorionic gonadotrophin from 8,00 to 0,000 I. U./L. urine (normal for the last part of pregnancy) less than 80 after delivery

Discussion

The cutaneous lesions corresponded from their beginning to termination closely to the description given by Hebra. The histological picture showed a Kogoj s pustule. The disease started late in pregnancy There was systemic disturbance which at first was slight, but later on the patient got so weak that, for a short time death was feared this condition might however have been due partly to intoxication with Avlosulfon. The fact that the efflorescences were pustules from their very beginning excludes herpes gestationis and dermatitis herpetiformis also the pustular form and the pustules differed by their firm consistency so much from the elements of the dermatosis of Sneddon and Wilkinson that this disease also can be discounted. Furthermore these conditions are excluded in a convincing way by the histological demonstration of the spongiform pustule of Kogoj. The début of the eruption on the abdomen and the weaker and far later attack of the hands and feet exclude acrodermatitis continua of Hallopeau. In so far as there were no ordinary clinical signs of psoriasis impetigo herpetiformis had to be diagnosed in preference to psoriasis pustulosa of Zumbusch.

With regard to treatment the following conclusions can be reached There was no help from Avlosulfon or Ledermycin. The external use of corticosteroids offered no advantage over zinc paste with 2 % sulphur Antex seems to have had a useful effect, partly because the patient recovered, and partly because

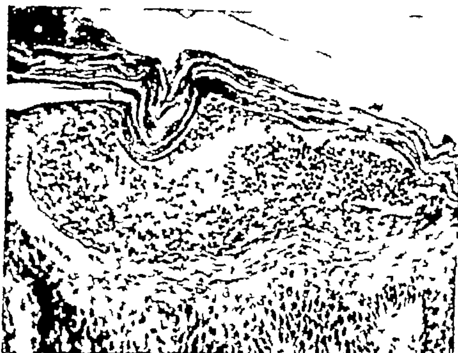


Fig. 3. The spongiform pustule of Kogoj. Hematoxylin and eosin stain × 160.

altered to a condition that must be described as erythroderma with extensive but minor lesions. After 9 days the dose of Antex was raised to 3000 I.U. daily. The general condition once more improved, rapidly. Seven days later the dose was increased to 6000 I.U. per day. The general condition had then been satisfactory for some time, and now a significant regression of skin lesions began, with islands of nearly normal skin appearing in many places. After a month's treatment with this dosage the lesions had disappeared leaving minimal erythema and slight hyperpigmentation. Antex was discontinued, a total of 217,500 I.U. had been given. There was no recurrence. One month later, at the expected date, normal labour took place and the patient gave birth to a healthy boy (4000 g).

When examined one month later and one year later the patient was healthy.

In addition to the internal medication mentioned, pyridoxine 40 mg three times daily was given from May 2. Furthermore, a zinc paste with 2 % sulphur was applied for the first five weeks. In this period, however, the right upper extremity was treated with a 1 % hydrocortisone ointment for one week and with Synalar ointment, 0.025 %, for two weeks. At no time was there any difference in the condition of the two upper extremities.

For the first month, the temperature was about 37° C. (max. 37.7° C.) and later between 36° and 37° C. Until May 26 the pulse-rate was about

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a distinct improvement set-in following each increase in dosage Including the present case Antex has been employed in 7 patients suffering from impetigo herpetiformis. Two of these who were non pregnant women were not benefited but the five others who were treated in connection with pregnancy became free from symptoms

SUMMARY

Impetigo herpetiformis is briefly surveyed The spongiform pustule of Kogoj has now been described by so many investigators that this histological finding although not specific must be con sidered so characteristic of impetigo herpetiformis that it must be regarded as a necessary supplement to the clinical signs for diagnosis. Reference is made to previous reports of treatment with Antex the gonadotrophic serum hormone of pregnant mares.

In addition a case is presented of impetigo herpetiformis in an 18 year-old woman starting in the seventh month of her first pregnancy The disease was not accompanied by hypocalcæmia The patient recovered during the administration of large doses of Antex

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Fig 1. Patient 9 days old Note rounded facies, broad flat bridge of nose, hypertelorism, low-set ears, and asymmetrical face.

Case History

The patient was girl aged six days. She was the only child of healthy parents. The mother was aged 27 years and the father aged 34 years. There was no known hereditary disease in the family The pregnancy was uncomplicated by other diseases. The mother had not been submitted to radiographic investigations nor did she consume any drugs during pregnancy

The patient was born in the Maternity Department of The Copenhagen County Hospital in Gentofte three weeks before the expected date of confinement. The birth weight was 2,500 g (5 1/ lb) and the length 49 cm (9") The Apgar score was 9 after minute.

At routine pediatric examination on the sixth day of life, the infant was found to be peculiar A chromosome anomaly was suspected and the infant was transferred to the Pediatric Department.

On admission, the child was found to be slender and hypotonic with hyper mobility of the joints The subcutaneous tissue was very loose. The face was singularly broad and nearly circular (Fig) with slight asymmetry on account of increased volume of the right side of the face. The base of the nose was

Hobolth, Niels, and Mikkelsen Margareta, Acta obst. et gynec. scandinav 44 572, 965

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MALADIE DE CRI DU CHAT [1]

BY

NIELS HOBOLTH AND MARGARETA MIKKELSEN

Since Moorhead (1960) described a clinically applicable technique for investigation of the chromosomes in human leucocytes numerous clinical syndromes due to anomalies in the chromosomes have been identified. The *majority of these conditions* can be diagnosed in the neonatal period on clinical criteria alone *e g Down s* syndrome.

In 1963 Lejeune *et al* published their investigations concerning a new syndrome due to absence of chromosome material in chromosome no 5 Since then, an increasing number of cases have been diagnosed (Boök *et al* 1963 Lejeune *et al.* 1964 Dumars *et al* 1964 Grouchy *et al.* 1964 Macintyre *et al.*, 1964 Punnett *et al*, 1964 Dyggve and Mikkelsen, 1965, and Hijmans and Shearin, 1965) The most striking feature of the syndrome is the peculiar cry which has given the syndrome its name

The authors had the opportunity to diagnose the present case during the neonatal period on the basis of the cry and the morphologic changes characteristic for the syndrome which consist of round face hypertelorism, epicanthus slanting anti-mongolic eyes strabismus micrognathia, low-placed ears, asymmetry of the face transverse creases in the palms and low birth weight in relation to the duration of pregnancy Older children show in addition, mental retardation, dwarfing and failure to thrive

[1] Presented in a modified form to the 394th meeting of the Danish pædiatric society on Jan. 13th 1965 and to the 395th meeting of the Danish society for obstetrics and gynæcology on March 19th 965.



Fig 3 Patient months old Note squint

induced to smile or fixate Pronounced hypotonia with very active reflexes and extended reflexogenic zones were observed There was tendency to foot clonus

The electroencephalogram was normal for the age of the infant.

Laryngoscopy (This was carried out in the Department of Otolaryngology The Copenhagen County Hospital in Gentofte and proved technically difficult) The epiglottis was rather small. The larynx appeared to be situated more highly than normal The laryngeal vestibule was very narrow and the impression was obtained that the mucosa was somewhat thickened although smooth and of normal colour The arytenoid regions stood out as two well-developed flat cushions At the base of the funnel formed by the vestibule, the two yellowish-red freely mobile ocal cords were seen. The sub-glottic space could not be seen

Cytological investigations Sex chromatin determinations were undertaken on cells from the mucosa of the cheek, stained with Feulgen, and were chromatin positive Chromosome investigation was undertaken on o ml capillary blood cultured by micro-method (F l d, 1963) Thirty metaphases were investigated In all of the cells examined, deletion of the short arm of chromosome in the 4-5 group was found The remaining chromosomes did not present any demonstrable abnormality (Fig 4)



Fig 2. Patient 9 days old. Note receding chin, clenched fists and dislocation in talo-crural joints.

broad and somewhat prominent, the orbital fissures were slightly slanting and antimongolic with slight epicanthus. A divergent squint was present. The lower jaw was poorly developed and deviating. Both feet were dislocated posteriorly at the talo-crural joints. Both thumbs were held folded in over the palms but the placing of the fingers showed no other peculiarities (Fig. 2) A typical transverse fold was observed on the left palm and, in the right palm, two parallel transverse creases were present.

The most characteristic feature in this patient was the cry which was very suggestive of the miaowing of a cat. Together with the appearance of the patient this made the diagnosis of *maladie de cri d chat* very probably

Care of the patient did not present any problems and the patient was discharged from the department after six days.

On re-admission for follow-up examination at the age of two months, the infant was found to be thriving reasonably well on a mixture of two parts cow's milk and one part water. The peculiar cry was still present. The face showed the same characteristic features as previously but to a slightly lesser extent (Fig 3) The axes of the eyes were divergent. The infant could not be

vealed that a pair of the 4–5 group are characterized by relatively early DNA synthesis while the other pair are late synthesizers. The deleted chromosome belongs to the early synthesizing pair and must be considered a chromosome no. 5 (German *et al.* 1964).

In any case the prognosis for the mental development of the child and for its survival is extremely poor. The oldest recorded child with this syndrome is 13 years old and has an IQ of 23 (Hijmans and Shearin, 1965). Early socio-medical measures are therefore indicated.

In one case, the disease developed as a sequel of balanced translocation between chromosome no. 5 and a chromosome in the 13–15 group in one of the parents (Lejeune *et al.* 1964). Such a translocation is only diagnosable by cytological investigation in the parents. Such an investigation was planned in the present case but, on account of lack of co-operation from the parents, it could not be completed. If a translocation is present, there is a considerable risk that subsequent pregnancies will result in defective infants and eugenic measures should be considered.

Hitherto, only a few cases of *maladie de cri du chat* have been diagnosed but the rapidly increasing rate at which the new cases are published indicates that the disease is more common than first supposed. As the disease can be diagnosed in newly born infants it is important that the medical staff of maternity departments have the condition in mind. It would also be of value to determine the frequency with which this chromosome abnormality occurs.

SUMMARY

A case of *maladie de cri du chat* diagnosed in a newly born infant is reported. The most striking feature of the disease is the cat-like miaowing cry. The syndrome is due to deletion of the short arm of a chromosome no. 5. The prognosis as regards mental development and survival is poor. The disease may be due to translocation in one of the parents. The parents should, therefore, be submitted to cytological investigation.

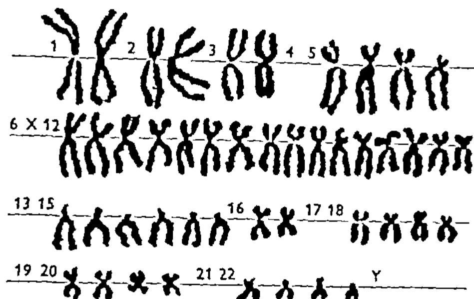


Fig. 4. Chromosomal pattern. Note deletion of short arm of chromosome nr 5.

Discussion

An increasing number of well-defined clinical pictures each of which is caused by a definite chromosome abnormality have become differentiated among retarded and deformed children. The most frequent of these is the excess of chromosome material as seen in Down s syndrome (21-trisomia) (Lejeune *et al* 1959) 17–18 trisomia (Edwards *et al* 1960) and 13–15 trisomia (Patau *et al* 1960) It has been recognized since 1963 that absence of parts of an autosome does not invariably lead to abortion but that the individual may survive for some years at any rate Absence of parts of the short arm of chromosome no 5 (Denver) causes not only severe psychic retardation and striking changes in the face but also changes in the larynx so that the cry becomes recognizably pathological The diagnosis may be suspected from the cry alone but it should always be confirmed by cytological investigation

There has been some discussion whether the deletion affects chromosome no 4 or 5. Autoradiographic investigations have re-

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AN UNUSUAL CHROMOSOMAL MOSAICISM IN OVARIAN DYSGENESIS

BY

MATS AHLGREN AND BENGT KÄLLÉN

The chromosomal constitution found in ovarian dysgenesis and Turner s syndrome is quite variable Apart from the classical finding of an XO sex chromosome constitution and a negative sex chromatin various types of mosaicism have been described by numerous authors (for reviews see de la Chapelle 1962 Lindsten 1963 Cou t Brown *et al* 1964) Some authors have found patients with gonadal agenesis and a chromosomal mosaicism of XO/XX_D where X_D represents a deleted X chromosome usually with the short arms deleted (*e g* Jacobs *et al* 1961 Lindsten 1963) De la Chapelle (1962) described two cases of XO/XX_D, where the deleted X in one case represented a long arm fragment in the other a possibly centric frag ment. Aspillaga *et al* (1964) described a new mosaicism XO/XX/XX_D the deleted X lacking short arms. This patient was of short stature and had diminished secondary sex charac teristics Of 130 cells counted, five had 44 chromosomes (arte facts) 97 had 45 chromosomes (XO) and 28 had 46 chromosomes, 16 being XX and 12 XX_D-cells Becker Hayles and Albert (1963) described an 8-year-old girl with short stature mental retardation, hyperextensibility of the fingers, and an unusual facial appearance Chromosomal analysis showed a mosaicism of XX/XX_D, the X_D representing a centric fragment.

In the present communication, a patient with dysgenetic ovaries

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Fig. 2. Karyotype of 47-chromosome cell. x marks extra chromosome possibly the short arm of a deleted X.

Laboratory data BMR −9 %. Meinicke test negative. FSH/urine > 40 MU/24 hrs. Oestrogens/urine < 25 MU/24 hrs. 17 KS/urine 7.8 mg/24 hrs. Vaginal smear: deficient oestrogenic activity.

Laparoscopy confirmed the presence of very small uterus from which small Fallopian tubes proceeded. Bilaterally at the site of the ovaries thin white streaks were seen. The right streak was approximately 2 cm in length and possibly contained two very small follicles. The left streak was smaller and without follicles.—A buccal smear for sex chromatin determination was taken, and also skin biopsy and repeated blood samples for chromosomal analysis. Because of the chromosomal pattern of the blood, laparoscopy was repeated to obtain biopsy from the gonadal streaks for chromosomal analysis.—Substitutional oestrogenic therapy has been started.

Cytogenetic data

The buccal smear showed a normal frequency of sex chromatin (> 40 %). Chromosomal analysis was made from peripheral blood on three occasions (method described by Källén and Levan 1962) on skin (method described by Hall, 1964) and

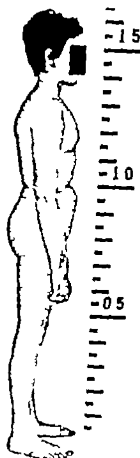


Fig. 1 Photograph of the patient.

is reported where a chromosomal mosaicism of Xx/XX/XXx was found x representing a probable short arm of a deleted Y

Clinical data

The patient, a female, born in 1943, was a hospital ward maid She was admitted to the Department of Obstetrics and Gynecology because of primary amenorrhoea. She felt quite healthy and had no previous illness of note. Her height and weight were normal (160 cm–57 kg) Her secondary sexual characteristics were underdeveloped very small breasts scanty pubic hair No cubitus valgus or webbed neck were found (Fig 1) The vulva and vagina were normal but uterus was rudimentary (probe cm) No ovaries could be palpated Roentgenological examinations showed the sella turcica and the adrenals to be normal, as was the ophthalmoneurological status

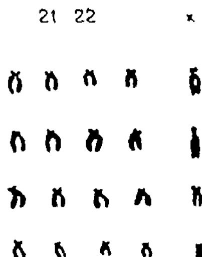


Fig. 4. Drawings of the small acrocentrics and the extra chromosome in four different cells. Note slightly varying size and morphology of the extra chromosome. All these cells were 46/Xx cells.

revealed a small fraction of abnormal cells, namely Xx and XXx cells, where x represents a small chromosome the approximate size of the small acrocentrics. It can be supposed to represent a deleted X chromosome, in which case it is probably the short arm.



Fig. 3. Karyotype of a normal 46-chromosome cell.

on a biopsy from a streak gonad (cultured in the same way as the skin) In skin and gonad tissue only XX chromosomes were found but relatively few cells were counted. In the blood however a mosaicism was found consisting of 57 46-chromosome cells and four 47-chromosome cells. Of the former 48 had an apparently normal female karyotype with 44 autosomes and XX nine had 44 autosomes one X and a small fragment. All four 47-chromosome cells had 44 autosomes XX and the fragment Figure 2 shows a karyotype of a 47-chromosome cell Figure 3 a karyotype of a 46-chromosome normal cell The size and appearance of the fragment varied slightly in different cells the average length corresponding approximately to that of the 21–22 group chromosome (Fig 4)

SUMMARY

This patient had a gonadal dysgenesis but a normal frequency of sex chromatin in the buccal smear Chromosomal analysis

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THE EFFECT OF PROSTAGLANDIN $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$, AND $F_{2\beta}$, ON THE HUMAN UTERUS AND THE FALLOPIAN TUBES IN VITRO

BY

F. SANDBERG, A. INGELMAN-SUNDBERG AND G. RYDÉN

In two previous papers (Sandberg *et al.* 1963 and 1964) the effects of prostaglandin E_1, E_2 and E_3 on the human uterus and the Fallopian tubes have been studied *in vitro*. These investigations have now been extended to prostaglandin $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$ and $F_{2\beta}$ (= $PGF_{1\alpha}$, $PGF_{1\beta}$, $PGF_{2\alpha}$, $PGF_{2\beta}$). These substances constitute two pairs of stereoisomers (Fig. 1).

Prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$ are naturally occurring substances found in human semen (Bergström and Samuelsson, 1962). $PGF_{2\alpha}$ has also been isolated from lung tissue and menstrual fluid (Bergström, 1964, Eglinton *et al.* 1963, Pickles *et al.* 1965). Prostaglandin $F_{1\beta}$ and $F_{2\beta}$ have been prepared synthetically by reduction of the keto group of PGE_1 and PGE_2 respectively (Bergström *et al.* 1962 a, b).

Prostaglandin $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$ and $F_{2\beta}$ were kindly supplied by Professor S. Bergström and Dr B. Samuelsson, Stockholm.

Material and Methods

The material was obtained at hysterectomies or salpingectomies. The Fallopian tubes were collected from 73 patients and the myometrial tissue from 56 patients. Only normal myometrial tissue and normal tubes were used.

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In two previous papers (Sandberg *et al.* 1963 and 1964) the effects of prostaglandin E_1, E_2 and E_3 on the human uterus and the Fallopian tubes have been studied *in vitro*. These investigations have now been extended to prostaglandin $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$ and $F_{2\beta}$ (=$PGF_{1\alpha}$, $PGF_{1\beta}$, $PGF_{2\alpha}$, $PGF_{2\beta}$). These substances constitute two pairs of stereoisomers (Fig. 1).

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Prostaglandin $F_{2\alpha}$, $F_{1\beta}$, $F_{1\alpha}$ and $F_{2\beta}$ were kindly supplied by Professor S. Bergström and Dr B. Samuelsson, Stockholm.

Material and Methods

The material was obtained at hysterectomies or salpingectomies. The Fallopian tubes were collected from 73 patients and the myometrial tissue from 56 patients. Only normal myometrial

CH CH₂ CH₂ CH₂ CH₂ CH₂ CH CH H H CH₂ CH₂ CH₂ CH₂ CH₂ CH₂ COOH

HO OH

PGF1α 9α 11α 15-trihydroxy prost 13 enoic acid

CH₃ CH₂ CH₂ CH₂ CH₂ CH CH CH H H CH₂ CH₂ CH₂ CH₂ CH₂ CH₂ COOH

HO OH

PGF1β 9β 11α 15 trihydroxy-prost 13 enoic acid

CH₃ CH₂ CH₂ CH₂ CH₂ CH₂ CH CH H H CH₂ CH CH CH₂ CH₂ CH₂ COOH

HO OH

PGF 9α, 11 15 trihydroxy prost 5,13-dienoic acid

CH₃ CH₂ CH₂ CH₂ CH₂ CH CH CH H H CH₂ CH CH CH₂ CH₂ CH₂ COOH

HO OH

PGF2β 9β 11α, 15 trihydroxy prost 5,13-dienoic acid

Fig. 1

After removal of the peritoneal covering, the tube was divided into four parts of equal length. The proximal segment was numbered 1 and the most distal (the infundibular) segment 4. Longitudinal muscle strips were cut from the corpus and transverse strips from the isthmus.

The experimental conditions and the methods for evaluation of the results obtained were identical with those described for

prostaglandin E_1, E_2 and E_3 in this journal (Sandberg et al 1963, 1964)

All four prostaglandins were given systematically in doses of 0.006–0.20 γ/ml.

Results

No difference in response could be demonstrated between specimens from different phases of the menstrual cycle. Therefore the results from the different phases have been summarized and listed in Tables I–IV

Prostaglandin $F_{1\alpha}$ (Table I)

I. Fallopian tube

The incidence of response is highest in the proximal segment of the tubes and decreases distally The effect observed in all segments is an increase in tonus and amplitude maximum (tonus + amplitude) whereas frequency and amplitude are unchanged.

II. Uterus

The incidence of response is low particularly in the corpus. The only demonstrable change is a slight increase in tonus in the corpus.

Prostaglandin $F_{1\beta}$ (Table II)

I. Fallopian tube

The incidence of response is low throughout but there is a weak stimulatory effect, as demonstrated by increase in tonus and amplitude maximum.

II. Uterus

The incidence of response is very low If a response occurs, it consists of a decrease in tonus, amplitude and amplitude maximum in the corpus. For the isthmus region the effect is limited to decrease in amplitude and amplitude maximum.

Prostaglandin $F_{2\alpha}$ (Table III)

I. Fallopian tubes

$PGF_{2\alpha}$ exerts a strong action on all segments of the Fallopian

PGF1α 9α 11α 15 trihydroxy prost 13 enoic acid

PGF1β 9β 11α 15 trihydroxy-prost 13 enoic acid

PGF2α 9α 11α 15-trihydroxy prost 5,13-dienoic acid

PGF2β 9β 11α 15 trihydroxy prost 5,13-dienoic acid

Fig. 1

After removal of the peritoneal covering, the tube was divided into four parts of equal length. The proximal segment was numbered 1 and the most distal (the infundibular) segment 4 Longitudinal muscle strips were cut from the corpus and transverse strips from the isthmus.

The experimental conditions and the methods for evaluation of the results obtained were identical with those described for

Table II. *In Vitro Effects of Prostaglandin $F_{2\beta}$ on the Longitudinal Musculature of the Fallopian Tubes from 28 Women and on the Uterine Musculature from 24 Women.*

Parameter		Fallopian tubes				Uterus	
		Segment 1				Corpus	Isthmus
Incidence of response		3/55 = 42 %	24/62 = 39 %	24/77 = 3 %	27/77 = 35 %	19/74 = 26 %	19/5 = 37 %
Change of motility pattern %		13	4	4	4	1	1
tonus	increase %	65	50	54	56	0	
	unchanged %	13	33	30	22	32	84
	decrease %	22	17	17	22	58	5
frequency	increase %	4	8	8	7	10	1
	unchanged %	74	54	75	8	41	56
	decrease %	22	38	17		40	53
amplitude	increase %	22	17	17	33	21	16
	unchanged %	44	50	45	45	10	5
	decrease %	34	33	38	22	69	79
ampl. max.	increase %	56	59	50	59	2	16
	unchanged %	1	4		19	0	16
	decrease %	34	37	29	22	79	68

The effect on the isthmus is similar but more pronounced, and in addition there is a decrease in frequency

tubes. There is a high incidence of response and an increase in tonus and amplitude maximum in almost all the experiments.

II. Uterus

The effect is less pronounced than on the tubes The typical action is an increase in tonus and amplitude maximum The muscle of the corpus is more sensitive than that of the isthmus.

Prostaglandin $F_{2\beta}$ (Table IV)

I Fallopian tubes

$PGF_{2\beta}$ has a weak action in all parts of the tube. The incidence of response is low The only demonstrable effect is a decrease in tonus and amplitude maximum

II Uterus

The action on the uterus is weak especially in the corpus the only recognizable effect being a slight decrease in amplitude and amplitude maximum.

Table I. *In Vitro Effects of Prostaglandin F on the Longitudinal Musculature of the Fallopian Tubes from 29 Women and on the Uterine Musculature from 11 Women.*

Parameter		Fallopian tubes				Uterus	
		Segment				Corpus	Isthmus
				3	4		
Incidence of response		53/65 =82 %	31/48 =65 %	40/72 =56 %	34/78 =44 %	12/32 =38 %	20/37 =54 %
Change of motility pattern %		21	13	5	9	0	20
tonus	increase %	89	87	90	77	58	5
	unchanged %	8	10	10	17	17	95
	decrease %	3	3	0	6	25	0
frequency	increase %	26	19	25	15	17	15
	unchanged %	59	81	72	76	58	40
	decrease %	15	0	3	9	25	45
amplitude	increase %	23	23	25	24	9	45
	unchanged %	53	61	58	70	33	30
	decrease %	24	16	17	6	58	25
ampl max.	increase %	89	87	85	91	42	50
	unchanged %	6	10	12	6	25	25
	decrease %	5	3	3	3	33	25

Table IV. *In Vitro Effects of Prostaglandin $F_{2\beta}$ on the Longitudinal Musculature of the Fallopian Tubes from 15 Women and on the Uterine Musculature from 17 Women.*

Parameter		Fallopian tubes				Uterus	
		Segment				Corpus	Isthmus
				3	4		
Incidence of response		16/29 = 55 %	5/27 = 55 %	23/34 = 68 %	17/46 = 37 %	38/86 = 44 %	31/42 = 74 %
Change of motility pattern %		25	13	17	24	11	23
tonus	increase %	9	7	5	6	8	0
	unchanged %	9	20	30	24	71	87
	decrease %	62	73	65	70		13
frequency	increase %	19	7	8	6	1	
	unchanged %	62	60	52	59	47	23
	decrease %	19	33	30	35	42	77
amplitude	increase %	6	7		24	1	3
	unchanged %	44	53	44	4	3	
	decrease %	50	40	35	35	58	87
ampl. max	increase %	0	7	5		3	3
	unchanged %	[illegible]	7	30	6	34	7
	decrease %	8	86	65	94	63	90

Table III *In Vitro Effects of Prostaglandin $F_{2\alpha}$ on the Longitudinal Musculature of the Fallopian Tubes from 10 Women and on the Uterine Musculature from 16 Women.*

Parameter		Fallopian tube				Uterus	
		Segment				Corpus	Isthmus
				3	4		
Incidence of response		32/32 =100 %	35/35 =100 %	37/40 ≈92 %	34/34 =100 %	56/97 ≈58 %	17/42 ≈41 %
Change of motility pattern %		9	9	8	6	18	18
tonus	increase %	100	100	100	100	84	59
	unchanged %	0	0	0	0	14	41
	decrease %	0	0	0	0	2	0
frequency	increase %	50	29	25	23	43	41
	unchanged %	50	66	75	77	54	53
	decrease %	0	5	0	0	3	6
amplitude	increase %	3	0	5	3	14	12
	unchanged %	44	63	68	70	41	47
	decrease %	53	37	27	27	45	41
ampl. max.	increase %	100	100	95	94	70	41
	unchanged %	0	0	5	6	30	47
	decrease %	0	0	0	0	0	12

vestigated. Thus, PGE_1 and PGE_2 have a stimulatory effect on segment 1 and produce a relaxation on the rest of the tube. $PGF_{1\alpha}$ and $PGF_{2\alpha}$ exert a stimulatory action on the tubes throughout. This means, that PGE_1 PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ have a similar action on the proximal part of the tube, but the first two substances have an opposite effect on the rest of the tube.

The effect of $PGF_{1\beta}$ is qualitatively the same as that of $PGF_{1\alpha}$ but weaker i.e. there is a stimulation of the tubes throughout. On the other hand $PGF_{2\beta}$ has a weak inhibitory effect similar to that of PGE_2.

Effect on the uterus

In general the effect of the PGF substances on the uterus is less pronounced than that on the Fallopian tubes, the incidence of response varying between 74 and 26 %. The α-substances have a stimulatory effect most pronounced in the corpus whereas the β-substances exert a weak inhibitory effect.

It may be observed, that among the naturally occurring substances PGF and $PGF_{2\alpha}$ have an action on the uterus opposite to that of the three PGE-substances. These findings are in agreement with observations made by Bygdeman and Eliasson (1963).

GENERAL SUMMARY

In two previous papers (Sandberg *et al.* 1963, 1964) and in the present investigation the effects of different prostaglandins on the Fallopian tubes and uterus have been tested by the Magnus-Kehrer technique using the same dosage range. Therefore the results obtained are directly comparable and have been summarized in Table V. The different parameters are evaluated in the previous papers and, concerning $PGF_{1\alpha}$, $PGF_{1\beta}$, PGF and $PGF_{2\beta}$, in Tables I–IV.

PGE_1 and PGE_2 exert a specific action on the Fallopian tube consisting of contraction of the proximal segment and relaxation of the rest of the tube. The other substances have a similar action on the different parts of the tube. PGE and $PGF_{2\beta}$ are inhibitory. $PGF_{1\alpha}$, PGF and PGF have a stimulatory effect, the strongest action being exerted by $PGF_{2\alpha}$.

Table V. *Summary of the Effects Exerted by Prostaglandins on the Fallopian Tube and the Human Uterus. A Stimulatory Effect Is Graded +++ ++ + and an Inhibitory Effect Is Graded -- - (-).*

PG	Fallopian tube Segment				Uterus	
			3	4	Corpus	Isthmus
PGE_1	++	--	--	--	-	--
PGE_2	+	--	--	--	-	--
PGE_3	-	--	--	--	-	--
PGF_1	++	++	++	++	+	o
$PGF_{1\beta}$	+	+	+	+	-	(-)
$PGF_{2\alpha}$	+++	+++	+++	+++	++	+
$PGF_{2\beta}$	-	-	-	-	(-)	-

Sandberg *et al.*, 1963
Sandberg *et al.* 1964
The present paper

Discussion

Biological activity on both uterus and Fallopian tubes has been demonstrated with the four PGF-substances investigated in graduated dosage, down to 0.006 γ/ml.

Effect on Fallopian tubes

The incidence of response shows quantitative differences. The most active substance tested is $PGF_{2\alpha}$ which gives 100 % response.

PGF_1 has a weaker action, the incidence of response varying between 82–44 % the highest response rate being in segment 1.

$PGF_{1\beta}$ and $PGF_{2\beta}$ have a still weaker action, the incidence of response varying between 68–31 %.

These results indicate that the naturally occurring substances exert the strongest biological action.

When PGF-substances are compared with the PGE-substances under the same experimental conditions and in the same dose range it is found, that the PGE-substances have a weaker action than $PGF_{2\alpha}$ as shown by a lower incidence of response. Thus $PGF_{2\alpha}$ exerts the strongest biological effect on the tubes. There are definite qualitative differences among the prostaglandins in-

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PROTON MAGNETIC RESONANCE STUDIES ON THE PHYSICAL STATE OF PROTEINS IN THE VAGINAL EPITHELIUM

BY

ERIK ODEBLAD

The present paper is a report of some examinations with nuclear (proton) magnetic resonance (NMR) on vaginal epithelial cell sediments. This investigation is a continuation of previous studies (Odeblad 1959 a, 1960, 1964). In those papers some physical properties of the aqueous phase of sediments of vaginal epithelial cells was studied. Strong arguments were presented indicating that the intra- and extracellular water fractions in vaginal cell sediments become separated at the time of ovulation when maximum oestrogenic stimulation of the epithelium occurs. This functional change of the cells is probably localized to the upper parts of the human vagina. In the present study the proton magnetic resonance signals of the aqueous phase are depressed by immersion of the cell suspensions in heavy water. The non-exchangeable protons in proteins and other compounds are mainly responsible for the observed proton spectra.

NMR is a non-destructive spectrographic technique capable of giving important information on the molecular physics of intact cells. In NMR spectrography the special lines are due to radiofrequency absorption by atomic nuclei in a magnetic field. The

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With the prostaglandins tested the action on the uterus is less pronounced than on the tubes, the effect being weakly stimulatory for PGF$_1$ and more pronounced for PGF$_{2\alpha}$. PGF$_{1\beta}$ and PGF$_{2\beta}$ have a weak inhibitory effect. PGE$_1$, PGE$_2$ and PGE give a pronounced relaxation.

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PROTON MAGNETIC RESONANCE STUDIES ON THE PHYSICAL STATE OF PROTEINS IN THE VAGINAL EPITHELIUM

BY

ERIK ODEBLAD

The present paper is a report of some examinations with nuclear (proton) magnetic resonance (NMR) on vaginal epithelial cell sediments. This investigation is a continuation of previous studies (Odeblad 1959 a, 1960, 1964). In those papers some physical properties of the aqueous phase of sediments of vaginal epithelial cells was studied. Strong arguments were presented indicating that the intra- and extracellular water fractions in vaginal cell sediments become separated at the time of ovulation when maximum oestrogenic stimulation of the epithelium occurs. This functional change of the cells is probably localized to the upper parts of the human vagina. In the present study the proton magnetic resonance signals of the aqueous phase are depressed by immersion of the cell suspensions in heavy water. The non-exchangeable protons in proteins and other compounds are mainly responsible for the observed proton spectra.

NMR is a non-destructive spectrographic technique capable of giving important information on the molecular physics of intact cells. In NMR spectrography the special lines are due to radiofrequency absorption by atomic nuclei in a magnetic field. The

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frequency f at which an absorption line, or magnetic resonance line occurs is given by *the basic equation*

$$f = \gamma H \qquad (1)$$

where γ is the magnetogyric ratio for the nucleus (here protons) and H is magnetic field. For a detailed discussion of NMR see e g the book of Andrew (1955)

The exact position of the resonance line of a sample depends on the chemical form of the atomic nuclei contained in the sample. The magnetic field H in equation (1) is given by

$$H = H_o (1 + \Sigma S \quad X + \sigma) \qquad (2)$$

where H is the applied bulk magnetic field in a permanent magnet or electromagnet X_n is the bulk magnetic susceptibility of the n th phase of the sample (of the order of -10^{-6} c.g.s. units) and S the shape factor of the n th phase the value being between 0 and 4 π depending on the geometrical relationships of the resonating nucleus and the phase boundary The constant σ is the internal diamagnetic shielding that is exerted by the electrons surrounding the atomic nuclei and is of the order of —30 ppm for protons. Both X and σ depend on the physical structure and chemical form of the sample Variation in σ gives rise to chemical shifts. If as is customary the shift of water protons are arbitrarily set as equal to zero some important chemical shifts can be seen from table *I Under nearly constant geometrical* and physicochemical conditions, as usually employed in the present investigation, chemical effects depending on σ govern the shifts in the NMR spectra If however paramagnetic substances e.g $CoCl_2$, are added susceptibility effects governed by the term sum

$$\Sigma S_n X$$

of equation (2) come into play CoCl was added in some experiments which are reported below

The magnetic resonance absorption is due to energy jumps between magnetic quantum *levels of the resonating nuclei* (see Fig 1 in Odeblad, 1959 b) If the atomic nuclei are subjected to strong resonance radiation the normal population difference between the magnetic levels of nuclei is removed. This effect

which is denoted saturation, will not persist when the strong radiation is interrupted. Some of the nuclei in the higher energy levels lose their magnetic energy to the surroundings and return to the lower levels. This process is denoted thermal relaxation and is characterized by a time constant, T The most molecular mechanisms causing thermal relaxation are provided by the rotational, translational or vibrational oscillations of neighbouring atoms or molecules, arising from the thermal (Brownian) movement.

Exchange of magnetic energy between two neighbouring nuclei also occurs. This process is also characterized by a time constant T The nuclear motions due to rotation, translation or vibration strongly govern the value of T as well as T but not in the same way For the dependence of T and T on atomic persistence times see fig. 5 in Odeblad, 1959 b. The more fixed the positions which the nuclei occupy the less are the relaxation times. At very long persistence times the value of T again becomes long while T remains at a low value.

If the "life-time of a nucleus in a magnetic level is short (*i.e.* T or T are short) the absorption line will be considerably broadened.

The signal area, that is, the integrated area under the graphically recorded absorption signal is, under otherwise equal conditions, proportional to the corresponding number of nuclei.

Clinical Material and Treatment of Samples

Samples of cervical secretion were taken from 11 healthy volunteers aged 17 21 years. Virgins only were selected to avoid contamination of the secretions with contraceptive agents, semen or other impurities. The subjects recorded their basal body temperature daily and were examined at least twice weekly during the period of study Phase determination was performed by examination of cervical secretion. At each examination about 0.5 ml of endovaginal contents was collected from the posterior fornix.

The material was immediately suspended in physiological saline solution and mucus was removed mechanically The cell sus-

pension was then centrifuged at 300 g for 10 minutes and the supernatant was removed. 2 ml of physiological saline made on heavy water was added and gentle stirring of the sample was per formed repeatedly during the following two hours. A second centrifugation was undertaken as above and the procedure was repeated once The heavy water contained 90 95 98 and 99 per cent D_2O in different runs. The D O solution contained a stand ard amount of Na^{22} for measurement of the sodium space in the sample. The sediment of the vaginal cells was transferred to nu clear magnetic resonance tubes and centrifuged at 300 g (time varying from 10 to 120 min.) to a constant volume The tubes were sealed by melting and were then introduced into the magnetic resonance spectrometer The seal was then broken. A capillary tube containing the calibration reference was introduced, and the spectra were recorded again

Three reference substances were employed for calibration, 1) a mixture of benzene and hexamethyltrisiloxane 2) benzaldehyde and 3) formic acid. By this choice different parts of the spectrum could be studied without interference of calibration lines (see table I)

Aliquots of 0 7 M CoCl in D O were added to the other sample tubes and centrifugation was performed. This served to displace the aqueous lines of the NMR spectrum by the para magnetic action of the Co ions and obtain clear identification of the lines belonging to aqueous phases

Instrumentation

Four magnetic resonance spectrometers were used in this in vestigation A) The spectrometer at the Nobel Institute of Physics as described by Lindstrom and Bhar (1956) with minor modifications The magnetic field was 4930 gauss and the proton frequency was 21 0 Mc/sec This spectrometer was used for routine investigations B) The spectrometer at Sabbatsberg Hospital (Odeblad 1961) having a magnetic field of 3910 gauss and operating at a proton frequency of 16 7 Mc/sec and a N frequency of 1 51 Mc/sec. This spectrometer was utilized for N H -double irradiation NMR studies C) The spectrometer at

Table I. Some Approximate Proton Magnetic Resonance Shifts in ppm from H O Reference for Different Chemical Groups or Compounds. (The Figures and 3 refer to the calibration system used, see text)

Group or Compound	Shift	Calibration
—CHO	—4 5	3
—NH_2	—3	
—C H_p, C_6H	—1 8	2
—OH, hydrogen bonded	—	3
H_2O		
—CH_2 —	2 5	
≡C —CH_3	3 5	
≡Si —CH_3	4 7	

Sabbatsberg Hospital having a field of 11,000 gauss and a proton frequency of 47 5 Mc/sec. D) The spectrometer at Sabbatsberg Hospital, having a field of 24,400 gauss and a proton frequency of 104.4 Mc/sec. Spectrometers C) and D) were used for detailed studies of proton spectra at higher separation than was possible using instruments A) and B)

Techniques for Measurement

The samples of endovaginal contents were introduced in the transmitter coil of the bridge The coil was placed in the magnetic field in a region selected to give the best instrumental resolution (0.05–0.15 ppm)

1) *Measurement of spectral positions.* The tube containing the sample (inner diameter of 0 5–1 6 mm) was introduced and the resonance spectrum was recorded. A small capillary was then introduced containing one of the three calibration substances mentioned. Each sample was measured with at least two of the three calibration substances. These methods of calibration enabled the determination of the position of the resonance line centres of the sample with a standard deviation of about ±0.1 to ±0.5 ppm. In each evaluation six to twel e readings were performed.

Some signals were recorded under saturation conditions.

2) *Measurement of T* The spin lattice relaxation time T was measured by observing signal growth after a strong (saturation) signal had been applied to the sample In several samples at least

pension was then centrifuged at 300 g for 10 minutes and the supernatant was removed. 2 ml of physiological saline made on heavy water was added and gentle stirring of the sample was performed repeatedly during the following two hours. A second centrifugation was undertaken as above and the procedure was repeated once The heavy water contained 90, 95, 98 and 99 per cent D O in different runs. The D O solution contained a standard amount of Na^{22} for measurement of the sodium space in the sample The sediment of the vaginal cells was transferred to nuclear magnetic resonance tubes and centrifuged at 300 g (time varying from 10 to 120 min.) to a constant volume The tubes were sealed by melting and were then introduced into the magnetic resonance spectrometer The seal was then broken. A capillary tube containing the calibration reference was introduced, and the spectra were recorded again.

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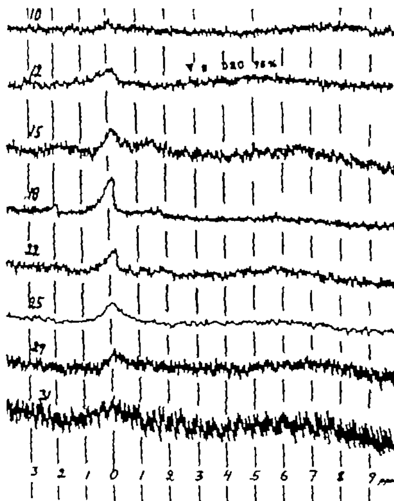


Fig. NMR spectra of protons in vaginal cell samples after exchange with 99 per cent D_2O as described in text, in the case M.O. on the days indicated in Fig. Abscissa scale: shift in ppm from H_2O reference. Ordinates: NMR absorption intensity. Note that the ordinate scales may differ between different tracings. The proton number per cm under the aqueous peaks are equal in all records (1.4×10^{20} protons).

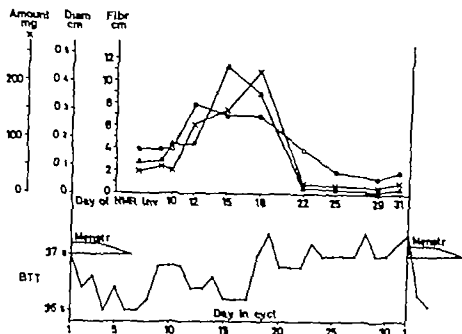


Fig. 1 Diagram of the cycle investigated in case M.O with the data under lying the phase determination. These data are Amount of cervical mucus diameter of external uterine os, fibrosity of cervical mucus, basal body temperature (BTT) and menstrual data. Ovulation seemed to occur around day 16 or 17 in this particular cycle.

tion method of Bloembergen (1948) was also used. The method errors were about ± 20 per cent.

3) *Measurement of T_2* The spin-spin interaction time T_2 was measured by the technique of Gabillard (1951) using repitition of signals and measurement of decay and growth of interference wiggles either on records or directly on an oscilloscope screen. The method error was about ± 15 per cent.

4) *Measurement of signal width* The signal width was measured either on the records containing the calibration signals or on separate recordings without calibration signals with sideband audiomodulation (Arnold and Packard 1951) An accuracy of about ± 10 per cent was estimated.

5) *Measurement of signal size* The signal areas were measured relative to the calibration signals. The signal size was then recalculated to the number of protons per cm of sample The method error was about ± 20 per cent.

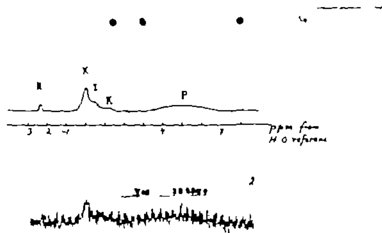


Fig. 3. A schematic diagram and record of proton resonance spectrum of vaginal cell sediment which has been subjected to exchange with 98 % heavy w ter. The approximate positions and shapes of the various peaks (N X, I, K and P) are shown in the diagram.

Results

The proton resonance spectra and relaxation times of vaginal cell sediments could be studied at 21.0 Mc/sec. during 11 complete menstrual cycles. The findings in one of these cycles is illustrated in Figs. 1 and 2. The results of the eleven cases are collected in table II. A total of 81 samples were investigated in this way. N^{14} H double irradiation NMR spectra were recorded in 8 samples in which the N peak appeared with sufficient amplitude. Proton spectra were studied comparatively at 16 7, 47 5 and 104 4 Mc/sec. in 12 samples, obtained from three additional virginal women (I L., T B, M. B.)

Five components have been resolved in the proton resonance spectra of vaginal cell samples after exchange with D O. The five components are denoted N, X, I, K and P (see fig. 3). The peaks X and I can be assigned to water and seem to be identical

Table II *Results of NMR Studies on Vaginal Cell Sediments after Exchange with 98 per cent D_2O Du = Duration Interv = Interval Po = Postmenstrual Mi = Midcycle Pr = Premenstrual Ov = Cycle with Ovulation Hy L = Hypoluteal Function An = Anovulatory cycle The estimated error contains both the standard error and the uncertainty of the evaluation of the records*

Case	Age Years	Menstr Du/Interv	Cycle Studied	Protons/cm³ in P Band (Units of 10^{20})			P Band Line Width /sec			P Band Shift, ppm from H_2O ref		
				Po	Mi	Pr	Po	Mi	Pr	Po	Mi	Pr
M. O	21	6/32	Ov	25	12	30	55	60	45	6.0	5.7	6.2
A. F	18	4/29	Ov	22	13	25	60	62	50	5.0	5.5	5.0
M B	19	4/30	Ov	25	14	27	55	55	45	5.5	5.5	5.5
K. B	21	5/27	Ov	33	18	30	50	60	55	4.5	5.0	5.5
Y A.	17	4/28	Ov	28	11	28	45	58	57	5.5	6.0	5.5
L T	17	5/28	Ov	31	16	44	55	60	47	5.2	4.5	5.0
A. L	18	5/28	Ov	46	19	37	53	58	45	5.2	5.2	5.5
S A	17	5/29	Ov	23	11	30	50	65	53	4.7	4.5	5.2
B B	2	5/28	Ov	33	19	23	60	63	50	5.7	4.7	5.2
			Average	30	15	30	54	60	50	5.3	5.2	5.4
			Estimated error	±3	±2	±3	±5	±5	±5	±0.2	±0.3	±0.2
M E	20	5/29	Hy L	30	16	20	56	62	48	6.0	5.8	5.5
Y V	19	5/28	An	28	15	19	63	60	52	5.5	5.5	5.7

Table III. *Shift and Line Widths of X I and K Peaks of Midcycle Samples*

Proton Frequency Mc/sec.	Shift, ppm X	I	K	P	Line Width c/sec X	I	K	P
16.7	—0.	+0.6	+1.8	4.0	1	3	16	57
47.5	—0.1	+0.6	+1.7	5.6	12	14	17	64
104.4	0	+0.7	+1.0	5.5	12	14	8	71
Standard error	±0.	±0	±0.	±0.4	±1	±	±2	±6

(Saunders and Wishnia, 1958 Jardetsky and Jardetsky 1957) Addition of cobalt ion does not displace this band. This is contrary to the observations of Philips Looney and Ikeda (1957) regarding the shift of aliphatic groups in alcohols in aqueous solutions containing Co ions. This discrepancy will be discussed below Although it seems reasonable to identify this band as arising mainly from protein protons, other compounds, such as mono- and polysaccharides, pyrimidines and purines in different linkages may also contribute.

The line width of the P band was as large as about 2–3 ppm. The relaxation time T of this broad signal was short, about 0.05–0.1 sec. The time T was very short and could not be exactly measured but was of the order of 0.003 sec. It must be emphasized that the P band may well be of complex origin and the figures given are a sort of weighted averages for the different partial contributions to this peak.

The peak N is located at about —2 ppm. It is usually very weak and has a width of about 0.5 ppm. None of the double irradiations indicated a triplet collapse suggestive of N^{1} bound protons Cyclic amino acids in free or bound state may give rise to the peak but its identification is not clear

Variations of the spectra occurred during the ovarian cycle. The aqueous peaks X and I behaved as stated in earlier papers, with a pronounced splitting into two peaks at the time of ovulation. The P band also exhibited variations the most constant being a considerable depression during midcycle.

The comparative recordings performed at 16.7 47.5 and 104.4 Mc/sec (Table III) showed a good separation of the X, I, K peaks and P band at the higher frequencies at the time of ovulation but

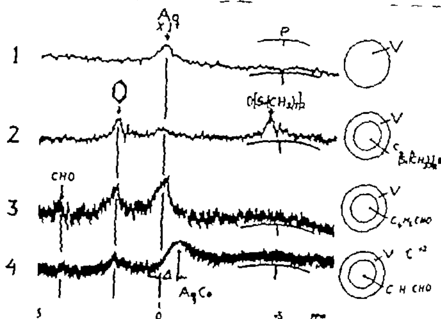


Fig. 4 Four records of case M.O at day 25 of the cycle (left) and the corresponding test tube arrangements (right) Record no. 1 was performed without calibration. In record 2 the internal tube contained benzene+hexamethyltrisiloxane and in record 3 benzaldehyde for calibration. Record 4 was performed with benzaldehyde calibration after the cell sediment had been exchanged with 0.7 M $CoCl_2$ in 98 % D_2O The trace indicates the susceptibility shift Δ of the aqueous phase due to the cobalt ions. Note that a shift of the P band does not occur V denotes vaginal epithelial cell sediments in the tube arrangement diagrams

with the aqueous peaks described earlier (Odeblad 1959, 1960) and are due to remaining extracellular (X) and intracellular (I) water The signal displacement by cobalt ions (fig. 4) gives strong support to this identification. Control experiments under identical geometry give the same direction and order of the shift for water upon addition of Co^{+} ions. The peak K has not been definitely assigned. It may also be a water fraction but it cannot be identified after addition of cobalt ions The broad peak or band, P is identified, by its shift of about +5 ppm from water reference to be due largely to protons in methyl or methylene groups, residing in protein compounds. This is in accordance with the interpretation of the NMR spectra of several purified proteins

dent loss of protons at midcycle under the P band is improbable, the cyclic depression of P band amplitude at the oestrogen peak may probably reflect a cyclic line broadening of the P band. The width of the broadened component, however is too large to be determined by the technique used.

The nature of the peak at —2 ppm is not clear It may be due to aromatic protons in some amino acids in proteins. Such amino acids have been identified in the vaginal secretion by Gregoire, Lang and Ward (1959) Attempts have been made to study the nature of this peak with N^{14}—H^1 double irradiation techniques but the results obtained are not consistent with the assignment of NH, or NH-protons.

SUMMARY

The non-destructive method of nuclear magnetic resonance spectroscopy has been applied to the study of the properties of the solid constituents in vaginal cell sediments. The positions of the proton resonances their sizes and their width were measured, and the relaxation times T and T as well as signal areas were determined. The proton frequencies used were 104.4, 47 5, 21.0 and 16 7 Mc/sec. Following exchange with 98 percent D O proton signals were identified at —2 (N) O (X) +1 (I) +2 (K) and about +5 to +6 ppm (P) from water resonance. The probable assignment of these signals are cyclic amino acids at —2 ppm, aqueous protons at 0 and +1 ppm (X and I peaks) and protein methyl and methylene protons at +5 to +6 ppm. The nature of the K peak at +2 ppm is uncertain.

The P signal at +5 to +6 ppm was the largest non-aqueous component, but was rather weak and broad. T was probably less than 0 05 sec and T about 0.003 sec., indicating considerable thermal proton mobility in the protein network. A cyclic alteration, probably involving a line-broadening at maximum oestrogenic activity (i.e a locking of proton sites) occurred in the P band.

Some evidence is presented that protein molecule assemblies of disc-shaped geometrical configurations may occur in the exfoliated epithelial cells.

no additional peaks could be found. The N resonance was too weak to be detected at 104.4 Mc/sec. Even at that high NMR frequency the P band did not split up into partial lines but was a single broad band indicating that highly mobile groups of high intensities in the protein molecules do not appear in the spectrum.

Discussion

The organic unexchangeable ("solid") matter which is visualized as the broad peak of *+5 ppm after D_2O exchange is characterized* by relatively short values of T_1 and T_2. The values are, *however not typical for a solid body in the physical sense* because the values of T_1 are too low and the T_2 values are too high to be representative for a solid state with fixed nuclear positions. As the protons in these constituents are essentially non-exchangeable the proton-carrying groups of the morphologic constituents of the cells must execute considerable thermal molecular movements in order to explain the observed T and T_2 values. Whether these movements are of rotational, translational or vibrational character cannot be decided at present. The shift of this peak is located at about +5—+6 ppm from water reference, which is some ppm higher than that which is customary for methyl and methylene protons. The reason for this high positive shift is not clear It may be associated with the shape of the protein molecule assemblies. The absence of shift to higher fields when Co ions were added is also remarkable and may arise from the shape (e.g disc shapes) of the protein assemblies in the living cells which leads to that the shape factors S contained in Equation (2) will have a negligible effect The effects discussed may therefore arise from disc-shaped protein molecule associations.

As the cell membrane is the site of pronounced cyclic changes, periodic changes in the broad component are expected. There seems to be a cyclically occurring depression of protein signal area at midcycle This depression may be due to immobilization of macromolecules by keratinization. The same considerations regarding molecular movements may also in part apply to hydration of organic molecules. Here however proton exchange effects come into play cancelling out the line broadening Since a tran-

sient loss of protons at midcycle under the P band is improbable the cyclic depression of P band amplitude at the oestrogen peak may probably reflect a cyclic line broadening of the P band. The width of the broadened component, however is too large to be determined by the technique used.

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